EXHIBIT 112

UNITED STATES DISTRICT COURT
NORTHERN DISTRICT OF CALIFORNIA

IN RE: ROUNDUP PRODUCTS LIABILITY LITIGATION
MDL NO. 2741
CASE NO. 16-MD-02741-VC

MONSANTO COMPANY'S NOTICE TO TAKE ORAL AND VIDEOTAPED DEPOSITION OF DR. MATTHEW ROSS
THIS DOCUMENT RELATES TO:

ALL ACTIONS

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VIDEOTAPED DEPOSITION OF
DR. MATTHEW ROSS

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APPEARANCES NOTED HEREIN

DATE: MAY 3, 2017
PLACE: MISSISSIPPI STATE UNIVERSITY
ALLEN HALL, 175 PRESIDENT'S CIRCLE
MISSISSIPPI STATE, MISSISSIPPI
TIME 9:33 A.M.

REPORTED BY: TODD J. DAVIS
BCR, CSR #1406, RPR

JOB NO. 123225

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Also Present: Eddie Nabors, Videographer
Dylan White, Esq. - MSU

INDEX

EXHIBITS:

(Exhibit No. 13-1 marked for identification.)
(Exhibit No. 13-2 marked for identification.)
(Exhibit No. 13-3 marked for identification.)

VIDEOGRAPHER: This is the deposition of Dr. Matthew K. Ross. This is the start of tape of DVD label number one of the videotaped deposition of Dr. Matthew K. Ross in Re Roundup Product Litigation. It is in United States District Court for the Northern District of California, Civil Action 16-MD-2741-VC.

The deposition is being held at Allen Hall, Mississippi State University, on May the 3rd of 2017, commencing at approximately 9:33 a.m.

My name is Eddie Nabors. I am the legal video specialist from TSG Reporting, headquartered at 747 Third Avenue, New York, New York. The court reporter is Todd Davis, also in association with TSG reporting.

Ask for counsel introductions on the audio portion, please.

MR. GRIFFIS: Kirby Griffis of Hollingsworth representing Monsanto.
MS. SHIMADA: Elyse Shimada of Hollingsworth representing Monsanto.
MR. TRAVERS: My name is Jeffrey Travers with the Miller Firm representing plaintiffs.
MS. WAGSTAFF: Aimee Wagstaff from Andrus Wagstaff in Denver, Colorado, representing the plaintiffs.
MR. WHITE: Dylan White representing Dr. Matthew Ross.
VIDEOGRAPHER: Will the reporter administer the oath, please.

MATTHEW K. ROSS, PH.D,
having been first duly sworn, was examined and testified under oath as follows:
MS. WAGSTAFF: So before we start, I would like to read something on to the record.
MR. GRIFFIS: Sure.
MS. WAGSTAFF: If you may. Just as an administrative matter, Mr. White and I are splitting a microphone which is clipped to a coaster between us, so we are proceeding hopefully that everything will be picked up by that microphone.
VIDEOGRAPHER: I am hearing you perfectly fine.
MS. WAGSTAFF: Excellent. Excellent. Secondly, Monsanto has requested that Dr. Ross's deposition to "explore the mechanism subgroups conclusion about glyphosate." They have requested this limited additional discovery, which the Court has allowed.
On April 18th, 2017, the MDL Court entered PTO 16, which said that, "Monsanto may subpoena Dr. Ross for 'fact deposition.'"

As such, plaintiffs will object to any expert testimony elicited by Monsanto or given to -- or given by Dr. Ross and will try to object as the questions are requested but present this general objection on the record before we begin.
MR. GRIFFIS: Anything else?
MS. WAGSTAFF: Nothing else. You may proceed.
MR. GRIFFIS: Yeah.
EXAMINATION BY MR. GRIFFIS:
Q. Yeah. I will address that.
Dr. Ross, have you been deposed before?
A. No. This is the first time.
Q. Okay. I am going to start by asking you to state your full name.
A. My name is Matthew K. Ross.
Q. And you are -- you have a Ph.D.?
A. I have a Ph.D.
Q. And in what, please?
A. It is in environmental toxicology, molecular toxicology.
Q. I'm going to go on and ask some more questions about your qualifications and do a

little housekeeping stuff like mark the legal documents that are going to be involved in this deposition.
We are going to be doing a number of things like marking documents, putting exhibit stickers on them, and then handing them to you. And the general format is that I'll be asking questions, and you'll be answering the questions.
I'm going to assume, if I ask you a question and you don't tell me that you haven't understood it, that you do understand it. And at times, your attorney may make an objection, or Ms. Wagstaff may make an objection.
If your attorney instructs you not to answer a question, then you're entitled to listen to him and not answer that question. Otherwise, it's your obligation to answer the questions that I've asked whether there's an objection or not.
Do you understand that, sir?
A. Yes.
Q. Okay.
MS. WAGSTAFF: I would object to the fact that he doesn't know when he doesn't understand you, but I understand your point.

MR. GRIFFIS: Sure.

The videographer has asked me to put on the record that his -- that although his instructions were to create a split screen video between me and you as a final production copy -- as going forward I have instructed him not to do that, but instead to make two videos. And we will clarify in post what we want done with those.

Presumably, we'll just take delivery of two videos, but in any event, his instructions were incorrect to that extent.

BY MR. GRIFFIS:

Q. I have marked as Exhibit 13-1 a subpoena to testify at a deposition in a civil action. It's called a notice of deposition. This was issued by Monsanto for your deposition here today, sir.

13-2 is a cross notice by the plaintiffs for the same deposition.

And 13-3 is a subpoena to produce documents, which I presume that you have seen before, sir. And I'm putting that into evidence because I will be asking some questions about it later and because the notice of the deposition

refers to it.

Have you seen any of those documents before, sir?

A. Yes.

Q. All three?

A. I have not seen this. No.

Q. Haven't seen the cross notice. But you have seen Monsanto's notice of deposition, and you have seen the original subpoena for documents to which you responded by producing some documents, correct?

A. Yes.

Q. Okay. And have you brought any -- other than your CV, which I'm about to mark as Exhibit 4 to this deposition, have you made any effort to gather documents for this deposition you didn't previously provide?

A. No.

Q. All right. Exhibit 13-4 is your CV.

(Exhibit 13-4 marked for identification.)

BY MR. GRIFFIS:

Q. Okay. That is a current copy of your CV, sir?

A. Yes.

Q. Would you please tell the jury your educational background?

MS. WAGSTAFF: Can I have a copy?

MR. WHITE: If you have another one, I'd also like to see.

Thank you very much.

A. So I received a bachelor of science degree in chemistry from UC Berkley in 1989. And then I received a Ph.D. in molecular toxicology from UC Irvine -- University of California at Irvine -- in 1998.

Q. Do you do bench research primarily, sir?

A. Yes.

Q. Would tell the jury what bench research is?

A. So the research I do is focused on analytical chemistry, bioanalytical chemistry, the study of how both environmental agents get metabolized in the body. In addition to how endogenous lipids get metabolized in the body.

Q. And what does bench mean in the terms of bench research?

A. Yes. Sorry. So bench research refers to work done in a laboratory under controlled

conditions. So we don't necessarily work with surveys or population surveys.

It is not epidemiological research. It's basic science done in a laboratory at the bench.

Q. And do you do work on experimental animals?

A. Yes.

Q. How much of your work is on experimental animals as opposed to in vitro?

A. I do mainly in vitro work. Mainly in cultured cells. Human cells, animal cells, and also in vivo studies in collaboration with other scientists at Mississippi State.

Q. And would you please explain to the jury in simple terms the difference between in vitro and in vivo. We just used both of those terms.

A. Sure. In vivo studies are studies that look at how a particular chemical may be metabolized within the body, within the human person, or in -- within an intact animal.

Those are studies that are performed so that you're looking at the whole system, the whole organism. In vitro studies are done in which cultured cells are used to study

various processes. It could be metabolism of a chemical. So in vitro is done in isolated cultured cells or what we call the subcellular fraction in which we obtain various parts of a tissue, but it is not the whole organism.

Q. And you mentioned both humans and animals when you described in vivo studies.

Do you perform studies in humans?

A. We use human cells. We use -- we use a cultured cell line that's derived from a -- from humans. We use tissues from humans. Primary cells that -- from actual human donors. So we use those types of materials from humans, yes.

Q. So those are all in vitro studies, though, not whole, intact human beings? They're done in --

A. Correct.

Q. -- essentially in a Petri dish?

A. Yes. In test tubes, Petri dishes.

Q. "In vitro" means in glass?

A. That's the Latin word.

MS. WAGSTAFF: I'm going to object to this, as it has nothing to do with the mechanisms, subverts, conclusions about glyphosate.

BY MR. GRIFFIS:

Q. With regard to in vivo studies done, have you done any in vivo studies in humans?

A. We -- let me see. As a bioanalytical chemist, I have looked at urine samples to measure pesticide metabolites.

Q. You have been involved as part of a team that was doing epidemiology work?

A. Correct.

Q. And what study or studies was that in connection with?

A. It was related to a study with permethrin.

Q. And what was the research group who was doing that study?

MS. WAGSTAFF: Same objection.

A. It was a research group here at Mississippi State.

BY MR. GRIFFIS:

Q. Have you been involved with the Agricultural Health Study?

A. I have been a member of their -- what do you call it? What is the right word? Their board that helps external advisory panel that -- that listens to some of their presentations.

Q. So you give scientific advice?

A. Correct.

Q. Have you performed any scientific work in connection with any of those studies?

A. No.

Q. Okay.

MS. WAGSTAFF: Same objection.

BY MR. GRIFFIS:

Q. Again, talking about in vivo studies only, sir, you told us that you don't do in vivo studies in humans. You don't run those yourself, at least, except to the extent that you may be involved in analyzing urine samples for pesticide residues, for example, as a part of someone else's epidemiology study.

Do you run in vivo studies in any species of intact animals?

A. In mice.

Q. Are you the primary researcher in those studies?

A. In collaboration with my colleague at Mississippi State.

Q. Okay. And you said that the majority of your work is in vivo work; is that right -- I'm sorry -- in vitro work?

A. The majority of my work, I would say, is done in vitro and in terms of bioanalytical chemistry of samples obtained from an intact animal like tissues or excreta from those animals.

Q. Have you done research on glyphosate?

A. No.

Q. That is true both before and after your involvement with working group 112, correct?

A. Yes.

Q. Okay. Working group 112 is the IARC group that looked into carcinogenicity of glyphosate and four other pesticides, correct?

A. Yes.

Q. Okay. I'm going to have a number of questions, obviously, today about your participation in IARC and how that came to pass, sir, and we'll turn to that in a moment.

First, I'd like to know, before you went to working group 112, before you went to Lyon, France, for that, did you know or had you met Christopher Portier?

A. I have never met him before volume 112.

Q. Didn't know who he was before?

MS. WAGSTAFF: Objection. This has nothing to do with the mechanisms, subgroups,

conclusions about glyphosate. Chris Portier is not even a monograph 112 member.
BY MR. GRIFFIS:
Q. Go ahead.
A. Did I know him? I knew -- I knew his brother. I did not know Christopher Portier. I had met his brother one other time.
Q. Okay. Before coming involved with working group 112, did you know Kurt Straif?
A. No.
Q. Before becoming involved with working group 112, did you know Phillip Landrican?
A. No.
Q. Did you know -- before becoming involved with working group 112, did you know Lauren Zeise?
A. No.
Q. Before becoming involved with working group 112, did you know Ivan Rusyn?
A. I knew of him. I knew of him, but I did not know him personally.
Q. You never met him?
A. I had never met him.
Q. Do you know how it was -- how it came to be that you were invited to participate in working group 112?

MS. WAGSTAFF: Objection. Calls for speculation.
A. I -- I think I became involved because of my experience in bioanalytical chemistry, in the area of toxicokinetics and metabolism, and extensive publications in organophosphate poisons.
BY MR. GRIFFIS:
Q. Do you know who whose -- who suggested your name to participate in working group 112?
MS. WAGSTAFF: Calls for speculation.
MR. WHITE: You can answer to the extent that you know.
A. I don't know.
BY MR. GRIFFIS:
Q. Were you ever told anything about why you were invited by anyone?
A. I don't recall.
Q. How did you learn that you were being invited to participate in working group 112?
A. I received an e-mail invitation from IARC.
Q. And about how long before the actual working group 112 convened in March of 2015 was that?
A. If I recall, I had an e-mail invitation

June 2014.
Q. And were there any rules imposed by the university on your consultation? Was there anything that you had to have cleared or approved before you could do that?
MS. WAGSTAFF: Objection. This is outside the scope of what Monsanto requested and what the judge allowed.
MR. WHITE: Again, only answer to the extent that you know.
A. The -- there was no stipulations. The only -- I only needed to get approval for international travel.
BY MR. GRIFFIS:
Q. Okay. So you got that approval, and you -- as far as you knew, there weren't any other requirements imposed by the university or clearances that you needed to get to participate in IARC working group 112?
MS. WAGSTAFF: Same objection.
A. There was -- no.
BY MR. GRIFFIS:
Q. All right.
(Exhibit No. 13-5 marked for identification.)

BY MR. GRIFFIS:
Q. Marked as Exhibit 5 an e-mail. And this is an e-mail that you produced to us during response to our deposition notice -- or our request for production of documents which is Exhibit 3.
This is from a Kathryn Forgie -- is that pronounced correctly -- who is a lawyer at Andrus Wagstaff, Ms. Wagstaff's firm, asking to meet with you.
And did you respond to this e-mail?
A. I don't -- I don't recall.
Q. You don't recall receiving the e-mail?
A. I do remember receiving this e-mail. I don't recall responding.
Q. Okay. Have you ever spoken to any lawyers other than Mr. White about your work on working group 112?
A. No.
MS. WAGSTAFF: Objection. Extremely vague. Any lawyers anywhere? What if he has friends that are lawyers.
MR. GRIFFIS: He has answered the question.

BY MR. GRIFFIS:
Q. Now, when did you first meet Christopher Portier, sir?
MS. WAGSTAFF: Objection. Again, outside the scope of the allowed deposition. Monsanto asked to explore the mechanisms, subgroups, conclusions about glyphosates. And Dr. Portier was not even on the monograph team.
MR. WHITE: Answer only to the extent that you know.
A. I met him the first time at Lyon, at the IARC meeting volume 112.
BY MR. GRIFFIS:
Q. At the introductory meeting?
A. At the first day of the meeting.
Q. And on the first day, there was an introductory welcome meeting where everybody got together, and there were some speeches; is that right?
A. I wouldn't call it speeches. Introductions of each member of -- and the panel.
Q. Did everyone sit down together, and people stood up and spoke a little bit about themselves or about one another by way of

introduction?
A. Yes.
Q. Did Mr. Portier introduce himself when he was talking about himself, or did anyone identify him as a current or former member of the Environmental Defense Fund?
MS. WAGSTAFF: Again, I am going to object -- have a standing objection to questions about Chris Portier. As I have said, before he was not even a member of the group, and he was not in the mechanism subgroup.
MR. WHITE: You're fine.
A. So he -- in the IARC list of participants, he had disclosed consulting for the Environmental Defense Fund. That was presented even before the meeting.
BY MR. GRIFFIS:
Q. You were given everybody's declaration of interests before the meeting?
A. Yes. There was a list of declaration of interests, and on that day, we had to sign if there had been any other conflicts of interest, potential conflicts of interest that needed to be disclosed on that very first day. There was a

form we had to sign.
Q. There was a supplemental declaration you filled out on the first day? How far before -- how long before the first meeting in Lyon did you receive other people's declaration of interests?
A. I believe -- if I recall, it was on the website of the IARC volume 112 meeting. When the participants are listed, their conflicts of interest were listed on that particular form that was on the website. I don't remember the time that showed up on the web, though.
MR. GRIFFIS: All right. Let's take five minutes so I can organize the next few exhibits.
VIDEOGRAPHER: Off the record at 9:55.
(A short recess was taken.)
(Exhibit No. 13-6 marked for identification.)
VIDEOGRAPHER: Back on the record at 10:07.
BY MR. GRIFFIS:
Q. Okay. Dr. Ross, I have marked as -- during the break, I marked as Exhibit 6 this deposition and handed you a copy of your declaration of interest for IARC working group

112, correct?
A. Yes.
Q. That's what that is?
A. Yes.
Q. Okay. On the third page of that document, in the box that says Nos. 5 through 6, you disclosed as one of your interests being on the advisory panel for the Agricultural Health Study; is that right?
A. Yes.
Q. And you wrote that you provided expertise on study design, data interpretation, and advice, correct?
A. Yes.
Q. When you were given information about other people's declaration of interests, including Mr. Portier's, did you see them in this form, or were you just given copies of other people's forms that they filled out?
A. I don't recall receiving their conflict of interests or declaration of interest in this form.
Q. In what form do you recall receiving it?
A. What is on the -- was on the website -- the IARC website for the meeting and the list

of -- the list of participants form that was at the meeting. Conflicts of interest were shown on that form.

Q. Okay. I want to mark this as Exhibit 7.

(Exhibit No. 13-7 marked for identification.)

BY MR. GRIFFIS:

Q. It is another document that you produced, sir, entitled -- headed "IARC International Agency for Research on Cancer," entitled, "Subgroup 4, working group members."

MS. WAGSTAFF: I'm just going to object that there's no Bates number on this or there's no production number or any sort of identifying number. But I assume it's authentic.

MR. GRIFFIS: It is.

BY MR. GRIFFIS:

Q. And this is a document that you received from IARC listing subgroup 4, working group members, sir?

A. It appears that way, yes.

Q. And you were on -- in working group 4 along with Dr. Rusyn as subgroup chair, correct?

A. Yes.

Q. Frank LeCurieux? Did I pronounce that right?

A. Uh-huh (affirmative response).

Q. Matthew Martin, William -- and Lauren Zeise. And invited specialist for subgroup 4 was Christopher Portier, correct?

A. Yes.

Q. And he's -- his affiliations here are listed only as retired; is that right?

A. Yes.

Q. Now, I've asked you about some of these people.

Did you know Mr. LeCurieux before joining working group 4?

A. No.

Q. Did you know Mr. Martin?

A. No.

Q. You met all of these people for the first time in Lyon; is that correct?

MS. WAGSTAFF: Objection to the form.

MR. WHITE: You can answer.

A. Yes.

MS. WAGSTAFF: You talking about in person that he met them before the meeting?

MR. GRIFFIS: Before being in Lyon is

what I'm asking.

MS. WAGSTAFF: Uh-huh (affirmative response).

A. I had not met them before Lyon.

MR. GRIFFIS: Okay.

(Exhibit No. 13-8 marked for identification.)

BY MR. GRIFFIS:

Q. Exhibit 13-8. I'm sorry. I shouldn't have said putting 13. We are putting "13-" in front of everything. But it's Exhibit 8 to this deposition. Sorry. Is a -- an overview of assignments for -- for group 4 for all of the substances being investigated; is that right?

A. Not only group 4. There --

Q. Yes, sir. All of the groups.

A. For -- for it appears to be all of the -- all of the four -- four groups.

Q. And would you quickly review for the jury what pesticides were being examined by working group 112?

MS. WAGSTAFF: Objection to scope.

A. First we worked on malathion, parathion, diazinon, tetrachlorvinphos and glyphosate.

BY MR. GRIFFIS:

Q. Now, do you know, sir, how those substances were selected to be reviewed by working group 112?

MS. WAGSTAFF: Speculation.

A. I don't.

BY MR. GRIFFIS:

Q. Did you learn at any time that glyphosate wasn't originally on the list?

MS. WAGSTAFF: Objection to foundation.

A. I had no knowledge of that.

BY MR. GRIFFIS:

Q. Okay. Did you learn at any time that Mr. Portier was involved in getting glyphosate added to the list?

MS. WAGSTAFF: Objection. Foundation.

A. I have no knowledge of that.

BY MR. GRIFFIS:

Q. Let's look at Exhibit 8, the assignments list, sir, and focus on glyphosate.

And this overview of assignments, what work -- what does it mean to be assigned a subsection?

A. So in my -- in my case, my responsibility was to review the toxicokinetic

data on glyphosate.
Q. And --
A. I was responsible for drafting the documents on the toxicokinetic data.
Q. And how far in advance did you receive your assignment with regard to glyphosate?
MS. WAGSTAFF: Objection to the form.
A. At approximately six months before the meeting, I received assignments.
BY MR. GRIFFIS:
Q. And what were you supposed to do in response to this those assignments?
A. We were charged with evaluating the published literature -- in my particular case, the toxicokinetic data on glyphosate in the published literature in publicly available literature and to synthesize a review of what is known regarding the toxicokinetics of glyphosate.
Q. And you prepared a written product from that, sir?
A. Yes.
Q. What was that written product?
A. It was the review of the toxicokinetic data regarding glyphosate.
Q. Was a draft of what ultimately became

the toxicokinetic data section of the IARC working group 112 monograph?
A. Yes.
Q. And did you have responsibility for writing sections for other substances, as well?
A. No.
Q. I see you listed under toxicokinetic data for tetrachlorvinphos?
A. Correct. So my charge was to write -- to review the toxicokinetic data for each of the five compounds that were being evaluated under volume 112.
Q. Okay. Before arriving in Lyon, in March of 2015, you were to prepare drafts of toxicokinetic data sections for malathion, parathion, diazinon, glyphosate, and tetrachlorvinphos; is that right?
A. Yes.
Q. And other people were doing the same for other sections, right?
A. Whatever was listed in this overview of assignments, that's -- that was their charge.
Q. When did you see other people's drafts in your subsection, in group 4?
MS. WAGSTAFF: Object to form.

A. We were asked to do peer review of certain sections. I did not do peer review of all the sections. We were assigned certain drafts to peer review before traveling to Lyon.
BY MR. GRIFFIS:
Q. How far in advance was that?
A. Approximately two to three months.
Q. With regard to glyphosate, which sections were you involved in reviewing?
A. Let me see here. I believe the one section that I peer reviewed for the meeting was 4.2.3 oxidative stress inflammation and the immune supression.
Q. Which was drafted by who?
A. Dr. Ivan Rusyn.
Q. Did you provide comments to that section?
A. Yes.
Q. During this process of preparing drafts and sending drafts, how were you sending and receiving drafts?
A. We used a server -- IARC server, IOPS system where we would upload drafts of the documents or peer reviews of a document that we needed to upload on to the server.

Q. And were you -- were you given a user name and password for IOPS?
A. Yes.
Q. And when you logged on to IOPS, what did you have access to from working group 112?
MS. WAGSTAFF: I'm going to object to the questions about drafts of IARC based on Judge Charbrio's (phonetic) order saying that IARC drafts are IARC property, immune from subpoena, pursuant to 22-USC-288-A, subsection B, and 919-F, sub 2B-43.
BY MR. GRIFFIS:
Q. Go ahead, sir.
A. Can you repeat the question?
Q. Sure. What did you have access to regarding working group 112 on IOPS?
A. So we could -- certainly, we would have access to our subgroup. We could access any of the documents that were being produced by the other subgroups if we wanted to read through them. So you could start looking at drafts before arriving in Lyon.
Q. Could you look at what studies had been tagged by your group and by other groups?
MS. WAGSTAFF: Same objection.

A. I don't recall.
BY MR. GRIFFIS:
Q. Did you participate in tagging studies for review?
A. For the toxicokinetic data, yes. I was charged with tagging some of the documents, yes.
Q. When you were given your assignment, had other people already tagged toxicokinetic documents for you?
A. No.
Q. So did you pretty much do all of the work of tagging toxicokinetic documents?
A. I believe I did.
Q. Was there a way for you to tag documents in other categories, or do you know?
A. I don't recall that. Whether I could tag documents in oxidative stress, I don't recall that.
Q. Okay. How -- if you wanted tay tag a -- and when we say tag a document, we're talking about a study?
A. Yes. A published study in the public -- in the publicly available literature.
Q. What was the process for tagging studies?

A. In my case, it was directly related to toxicokinetic data, whether it described the absorption, distribution, metabolism, and excretion of glyphosate.
Q. Yes, sir. I'm asking something a little bit different.
Let's say if you had a study in mind that you wanted to tag. What would you actually do on the computer to tag it?
A. We would evaluate the abstracts. And if it clearly looked relevant, we would tag them right then and there. If we were uncertain about the relevance, I would try to get access to the copy of the full article to -- if the abstract wasn't revealing to me enough about the relevance of the article, I would try to get a copy of the actual -- the full article to include it or not include it.
Q. Was there a box to check to tag or not tag documents?
A. We had some mechanism of including or excluding the study in our evaluation.
Q. Now, there was also an online system called the HAWC, H-A-W-C; is that right?
A. Yes.

Q. Okay. And were you given a user name and password for HAWC?
A. Yes.
MS. WAGSTAFF: Same objection. IARC drafts and work product.
BY MR. GRIFFIS:
Q. What was the difference between what you were doing on IARC and what you were doing on HAWC?
A. I don't recall. I don't recall the difference. I think the IOPS system was simply a way to upload documents, and HAWC was the software that allowed us to tag documents to include or exclude an evaluation.
Q. So the tagging would have actually been taking place on HAWC, and if you wanted to share a document with the group, it would go through IOPS; is that right?
A. I don't recall the specifics of sharing PDFs of the actual studies. I don't recall.
Q. Okay. Did HAWC also have tools for doing data analysis?
A. Not for the toxicokinetics.
Q. You didn't see any data analysis modules on HAWC for working group 112?

A. I don't recall ever seeing those.
Q. Did you see any modules that were -- could be used to manipulate or generate statistical analyses of data?
A. No.
Q. Okay. Did HAWC have capacities that you were aware of to process or store or display data from studies in any way?
A. Not that I am aware of.
Q. Okay. So if I want to summarize the IOPS and HAWC so perhaps we can move on from it, from what you used those two systems for, then, would have been, one, to tag literature in your assigned areas for these various documents, i.e., toxicokinetic data; and, two, with regard to the IOPS system to upload your draft sections on toxicokinetics and to download any drafts that you wanted to read that other people had done.
Is that right?
MS. WAGSTAFF: Objection. You're testifying. That record speaks for itself.
A. The HAWC system was used for tagging studies for inclusion or exclusion. And IOPS was used for uploading documents, and we could access other -- other documents in the -- in the IOPS

system, other drafts.
BY MR. GRIFFIS:
Q. And was there anything else that you used either of those systems for other than what we just talked about?
A. No.
Q. Okay. Explain to the jury what toxicokinetics is, please.
A. Toxicokinetics relates to the absorption, distribution, metabolism, and excretion of a particular chemical in the body.
Q. So it's -- is it a fair summary to say how a chemical moves through the body from start to finish?
A. Yes.
Q. Okay. And toxicokinetics were the only sections you were responsible for before showing up in Lyon; is that right?
A. Yes.
MS. WAGSTAFF: Object to the form.
BY MR. GRIFFIS:
Q. Would you have reviewed studies in the other working group 4 subareas like receptor mediated effects, altered self proliferation, cancer suseptibility data, et cetera, other than

toxicokinetics, of course, before showing up in Lyon?
A. I was charged with peer reviewing the oxidative stress drafts before showing up in Lyon.
Q. Did you review the oxidative stress drafts for all of the substances?
A. I don't recall.
Q. Did you have different assignments than oxidative stress from some of the other substances?
A. I did. I -- yes.
Q. Do you recall if you had one assignment for each substance -- one peer review assignment for each substance?
A. I don't recall.
Q. Okay. Do you recall about how many peer review assignments you had total?
A. I can't remember exactly. Maybe three, maybe four.
Q. How many hours of work do you think you put into the peer review of glyphosate oxidative stress section?
A. Two to three hours.
Q. And what did that -- those two to three hours of work entail?

A. Reading the draft and providing comments on the draft document.
Q. Did you review any of the studies?
A. That were in the draft?
Q. Yes, sir. In those two to three hours, did you actually read any of those studies that were cited therein?
A. I don't recall.
(Exhibit No. 13-9 marked for identification.)
BY MR. GRIFFIS:
Q. Dr. Ross, I marked as Exhibit 9 a working group 112 meeting timetable that you produced, and that is what's in front of you; is that right?
A. I didn't produce this. You mean -- what do you mean produced?
Q. I'm sorry. I'm being a lawyer when I say "produced." We asked you to provide us with documents that IARC -- and you turned those documents over, and I'll ask you a little bit more about how you did that exactly. But we ultimately received documents from you, and this is one of the documents that we received.
So this is one of the documents

that you provided to us in response to our document request which is Exhibit 3; is that right?
A. Yes.
Q. Okay. And this is a timetable that I take it you received from IARC for working group 112, right?
A. Yes.
Q. Okay. And it shows activities from the evening of March 2nd through the afternoon of March 10th of 2015, right?
A. Yes.
Q. Okay. And on March 2nd, the only activity is an evening meeting -- an evening planning meeting between meeting chairs and subgroup chairs only, correct?
A. That's correct.
Q. Were you involved in that?
A. No.
Q. Okay. Would you have first started meeting people on the 3rd?
MS. WAGSTAFF: Object to the form.
A. Yes.
BY MR. GRIFFIS:
Q. Do you remember when you got into Lyon?

A. March 2nd.
Q. Okay. And did you not head over to IARC until March 3rd?
A. Correct.
Q. All right. And when did you leave Lyon?
MS. WAGSTAFF: I am going to object to these questions. This has nothing to do with the requested discovery of the mechanisms, subgroup conclusions about glyphosate -- when he arrived and when he left Lyon. You're just badgering the witness.
BY MR. GRIFFIS:
Q. Go ahead, sir.
A. Wednesday, March 11th.
Q. Okay. And when you talked earlier about introductions, meeting people, was that during the opening session of March 3rd, sir?
A. Correct.
Q. Now, there were -- there were a number of subgroup sessions listed on the 3rd, 4th, 5th, 6th, and 7th of March.
What is a subgroup sessions?
A. These are the times where each subgroup meets together to evaluate the drafts.
Q. And there's also evenings of the 3rd,

4th, 5th, and 6th, something called a coronating meeting for the co-chairs and subgroup chairs, correct?
A. Yes.
Q. Were you involved in that?
A. No.
Q. Okay. And so the subgroup sessions -- there were 11 of them that you attended; is that right?
MS. WAGSTAFF: Objection. Foundation. Doesn't even show how it was followed.
A. There are 11 subgroup sessions listed on this.
BY MR. GRIFFIS:
Q. Did you go to all of them?
A. Yes.
Q. Were there subgroup sessions that were held that weren't listed on this on the itinerary?
A. We would meet to -- if there was an important topic that needed to be raised within the subgroup outside of this 11.
Q. What percentage of the working group 4's time was spent on glyphosate as opposed to one of the other four pesticides under review?
A. So we had five compounds. I would

estimate we spent 20 percent of them the time.
Q. About evenly divided?
A. Yes.
Q. And what percentage of that time would you have spent talking about the issues of genotoxicity and oxidative stress?
A. In the subgroup sessions a lot of the time was spent on those issues.
Q. Lot of the glyphosate time would been spent on those two issues?
A. Correct.
Q. Okay. All right. And who was involved on behalf of group 4 in coordination meetings?
A. You are referring to the meeting at the end the coordination meeting for cochairs?
Q. Meeting at the end of early of days the 3rd, 4th, 5th, 6th. That says coordination meeting for the cochairs and subgroup chairs?
A. That would have been our subgroup chair of group 4.
Q. Dr. Rusyn?
A. Dr. Rusyn would have been participating in those.
Q. Do you know if Chris Portier was at those?

A. I don't believe so. He -- no. I don't think he was.
Q. Did you witness people going off into those meetings, or were you off doing your own thing by then?
A. No. I didn't witness.
Q. All right. Mr. Portier is listed as an invited specialist for group 4. That's in the Exhibit 7, I believe, sir.
What was your understanding of what he was an invited specialist for, for group 4?
A. So Dr. Portier is a biostatistician, and he was invited as a specialist to help peer review the tox cast data that was being presented.
Q. For any other purpose?
A. Not that I am aware of.
Q. Did he speak to your group, address your group about issues other than tox cast data?
A. He acted as a peer reviewer.
Q. If he were to give an opinion to the group on the subject of biostatistics and a analysis -- a reanalysis of biostatistics, would you be qualified to evaluate the scientific merit of that opinion?
MS. WAGSTAFF: Objection. Calls for

speculation and hypothetical. You can't just say any opinion Chris Portier gives.
A. I'm not a biostatistician. It's not my area of expertise.
BY MR. GRIFFIS:
Q. Okay. So if Chris Portier or another biostatistician gives a biostatistics opinion, you wouldn't be qualified as a peer to second guess that opinion.
Is that fair?
MS. WAGSTAFF: Objection. Hypothetical. Calls for speculation. You don't know what opinion you're talking about.
A. Yeah. It would depend on the conversation. Clearly, I can understand the importance of statistical significance and whether an effect is statistically significant, but my area of expertise was on toxicokinectics.
BY MR. GRIFFIS:
Q. You were focused on the toxicokinetics during these conversations and not on biostatistics or the other areas listed.
Is that fair?
MS. WAGSTAFF: Objection. Misstates the record. That's not what the deponent said.

A. My main responsibility was the toxicokinetic sections.
BY MR. GRIFFIS:
Q. Were you asked by IARC to read their preamble.
Do you know what I'm talking about when I say the preamble?
A. Yes. And I did read it.
Q. Okay. You were asked by IARC to read that?
A. Yes.
Q. Okay. As part of your preparation for to participate in working group 112?
A. Correct.
Q. What was your understanding of the purpose for your review of the preamble and how it was to guide you if it was?
A. Repeat the question.
Q. Yes, sir. What was your understanding of -- I will make it a little simpler.
What was your understanding of why you were being asked to review the preamble?
A. It is a guiding document for how the meeting is run, how we evaluate the information, the data that we asked to review. And it provides

a rubric for how the classifications are made.
(Exhibit No. 13-10 marked for identification.)
BY MR. GRIFFIS:
Q. Marked as exhibit 10 is a copy of the IARC preamble.
That is what you reviewed, sir?
A. This says 2006. I don't know if there was a -- what -- if this was the actual document. But the preamble -- whatever they have on their website -- they have it on their website -- is what we read. And they had this a hard document -- a hard copy on the first day of the meeting.
Q. Okay. So everybody would have to read it in advance, and everyone was also given a hard copy on the first day; is that right?
A. Correct.
Q. Okay. And one thing you just told me earlier is that this provided a rubric for your evaluation.
Would you explain what you mean by a rubric for your evaluation?
A. In terms of mechanistics subsection, there were key characteristics of carcinogens that

were evaluated. There's ten key characteristics. And we were asked to provide -- as a subgroup to provide qualitative descriptors of strong, moderate, or weak in terms of the evidence for each particular character -- key characteristic.
Q. Okay.
A. It...
Q. Sorry. Were you done?
A. Yes.
Q. Okay. So there were ten key characteristics.
And these are different categories of mechanism; is that right?
A. These are -- yes. Different categories, different mechanisms by which a carcinogen may act to cause human cancer.
Q. Do you know the source of those ten characteristics?
A. There is an environmental health perspectives study or paper that lays out the ten key characteristics. It is in the published literature.
Q. Okay. Do you know when that was published?
A. I believe it was in 2016.

Q. Okay. Do you know if it was published before or after your working group met?
A. It -- this is -- the formal document came out in 2016, but the characteristics were listed on the IARC website where somewhere IARC had a listing of these key characteristics that the subgroup was charged with evaluating.
Q. Do you know if those had been submitted to the publication in peer review process before working group 112 met?
A. I don't recall that.
Q. It was published in 2016.
You don't know when might been peer reviewed; is that right?
A. I don't --
MS. WAGSTAFF: Objection. He said that the ten key characteristics were listed on the IARC website. That has nothing to do with whether or not it was published. Because some author decided to turn it into a publication is irrelevant.
BY MR. GRIFFIS:
Q. And the classifications that you could give for each of the ten characteristics were -- repeat them, please.

Weak?
A. The qualitative descriptors?
Q. Yes. The qualitative descriptors.
A. Those were weak, moderate, or strong. And those come from the preamble.
Q. Okay. And so for each of the ten -- so any study would be divided into one or more of the key characteristics and used to evaluate mechanism under the rubric of that characteristic; is that fair?
MS. WAGSTAFF: Objection. Misstates the testimony.
A. There -- the papers that were related to genotoxicity -- the evidence based on genotoxicity or oxidative stress were bin -- so papers within those -- since those are the two characteristics that were deemed strong, those papers were within each of those bins.
BY MR. GRIFFIS:
Q. Okay. And so it would be sorted into the ten bins. And then as to each bin, the group was asked to conclude one of three things: Weak, moderate, or strong; is that right?
MS. WAGSTAFF: Objection. Misstates the testimony.

A. We didn't -- if the evidence was weak, we didn't -- we didn't have to spend a lot of time on that evidence. If it was strong, there was a clearly -- in the monograph, there was a statement to that effect, that the evidence was strong based on the evidence -- the papers were deemed important.
BY MR. GRIFFIS:
Q. Well, all I'm asking you right now, though, is your three choices were weak, moderate, and strong, right?
A. Those were our descriptors.
MR. GRIFFIS: Okay. Take a break at this point.
VIDEOGRAPHER: All right. Off record at 10:44 a.m.
(A short recess was taken.)
VIDEOGRAPHER: Back on record, 10:56.
BY MR. GRIFFIS:
Q. Dr. Ross, you told us earlier that your group divided its time pretty evenly among the five substances that were being reviewed, including glyphosate.
So you estimated about 20 percent of your time was spent on glyphosate, right?

A. We spent approximately equal time on all compounds.
Q. So is it fair to say that your working group, when it was working together, did the equivalent of about a day's work on glyphosate during work group 112?
MS. WAGSTAFF: Objection. Misstates the record. Who knows what a day's work means.
A. We had several days on glyphosate.
BY MR. GRIFFIS:
Q. And those same days were also spent on other substances, right?
A. There were other substances discussed in a given day.
Q. When I say one day's work, I didn't mean to suggest to you set aside one particular day to focus on that and moved on. I was trying to get a sense of, over this week, how much total work went into it? Was it about a day's work --
MS. WAGSTAFF: Object to the form.
BY MR. GRIFFIS:
Q. -- divided over multiple days?
MS. WAGSTAFF: Same.
A. It was more than one day's work.

BY MR. GRIFFIS:
Q. Okay. There were --
A. Several days work.
Q. How many days -- during how many of these days was work done on? I am looking at Exhibit 9, the timetable.
A. It doesn't say which -- for each subgroup sessions, it doesn't say which compounds we were working on at the time.
MS. WAGSTAFF: I'm going to object also -- Dr. Ross said they met at night when needed.
BY MR. GRIFFIS:
Q. So there was actual work done on March 3rd, on March 4th, on March 5th, on March 6th, correct?
A. Subgroups, 3rd, 4th, 5th, and 6th, 7th, we met in subgroup. Those were the times we were meeting in subgroup. There was work being done on Sunday. There was reading over drafts. There was work being done in the evening.
Q. How many total -- on how many total days during your time in Lyon was work being done on glyphosate?
MS. WAGSTAFF: Object to the form.

A. I don't recall how many days. There were several days we were meeting to -- with each of the compounds. And I don't recall the exact number of days that we've -- that we were on glyphosate.
BY MR. GRIFFIS:
Q. Well, the 3rd through the 10th is seven days. Fair?
A. Yeah. Yeah. Eight days if you count Tuesday.
Q. Okay. Do we count Tuesday? Was substantive work done on Tuesday?
A. Yes.
Q. Okay. Eight days total were spent in Lyon doing this work, right? Five substances were involved. And you told us your work was divided evenly?
MS. WAGSTAFF: Going --
BY MR. GRIFFIS:
Q. Can we conclude that the amount of work done on glyphosate was eight divided by five?
MS. WAGSTAFF: I'm going to object to this question on the suggestion that all the work was done in Lyon. He has testified numerous times that months of work were put

into this prior to the meeting.
A. We had our assignments six months before the meeting. So there was six months of work being done before we met in Lyon.
BY MR. GRIFFIS:
Q. Yes, sir.
You testified you worked on the toxicokinetic data and that you did a peer review that took two to three hours of work. Let me -- let me clarify something. It's a point I made a little earlier, but I didn't ask you in that last question.
When the group was working together, in whole group work together, the total amount of time you could spent on glyphosate, given your testimony, working together, would have been eight days divided by five substances; is that right?
MS. WAGSTAFF: Objection. Misstates the testimony.
A. Repeat the question now.
BY MR. GRIFFIS:
Q. Okay. And let's first address the work before you showed up.
It would not have been the case

that the entire group was focusing on oxidative stress or the entire group was focusing on genotoxicity or the entire group was focusing on any other of the ten characteristics that were binned with regard to glyphosate prior to meeting in Lyon; is that right?
MS. WAGSTAFF: Objection. Dr. Ross can't testify to what other panelists were focusing on.
A. My focus was on the toxicokinetics. That is what I was responsible for. And I was responsible for peer reviewing the draft on oxidative stress prior to the meeting.
BY MR. GRIFFIS:
Q. So prior to the meeting, you spent about two to three hours peer reviewing the oxidative stress draft.
And other than that, you were focusing on solely toxicokinetic data prior to showing up at IARC, right?
MS. WAGSTAFF: Objection. Misstates testimony.
A. I was working on peer reviews of other compounds -- others than were not related to glyphosate.

BY MR. GRIFFIS:
Q. Okay. I do mean to limit myself to glyphosate in that question.
A. So the peer -- when I say the peer review takes two to three hours, that's just the reading of the document. That does not include the amount of time in responding point by point to the author.
Q. How much time did you take doing that?
A. Must have -- oh, at least a day. And I did -- I did look up some methodology papers and some of the -- some of the citations I did look up what type of method they were using for their oxidative stress measurements. So that would take some time, as well.
Q. How much additional time?
A. That probably would take about an hour to two hours look at that information.
Q. So about a day and half total work for the peer-review process work for oxidative stress?
A. Roughly, yes.
Q. Okay. And you've -- you were not focused on the genotox prior showing up in Lyon; is that correct?
MS. WAGSTAFF: Objection to the form.

A. I did not review the genotox --
BY MR. GRIFFIS:
Q. You weren't included -- sorry.
A. No.
Q. You weren't included in any discussions by the rest of the working group on genotox or oxidative stress or anything else that took place before showing up in Lyon; is that right?
MS. WAGSTAFF: Object to the form.
A. The oxidative stress I had a -- I had peer reviewed the draft before attending Lyon.
BY MR. GRIFFIS:
Q. Yes, sir. But the entire working group was not exchanging communications about the oxidated stress or genotox or anything else as a group prior to showing up in Lyon; is that right?
A. In terms of myself, I wasn't sharing except for the peer review of the oxidative stress. There may been others who had interactions before the meeting, but I am not aware of that.
Q. Can't have been the whole group because you were part of the whole group, and you didn't see it?
A. As a group, we met in Lyon to go through

the drafts. That was the first time we were all together.
Q. Okay. And as a group, the total amount of time you could have spent was about eight days divided by five substances on glyphosate; is that fair?
MS. WAGSTAFF: Object to form. He stated that they spent 20 percent of the subgroup session. He also stated they worked at night and evening. He never said that was 20 percent.
A. We -- there were some nights we would work on -- I would work on one compound through the night, glyphosate. So I can't -- I don't know the exact number of hours on glyphosate --
BY MR. GRIFFIS:
Q. Okay.
A. -- during the eight days.
Q. There were plenary sessions in addition to the subgroup sessions, correct?
A. Yes.
Q. What is a plenary session?
A. Where all of the four subgroups come together.
Q. And the first plenary session was on the

morning of Wednesday, March 4th, and it was called evaluation criteria, right?
MS. WAGSTAFF: I'm going to go ahead and object to questions about plenary sessions, as Monsanto had an employee there. And, also, the request for this deposition was to "explore the mechanism subgroup's conclusions about glyphosate."
A. The question -- repeat your question.
BY MR. GRIFFIS:
Q. Yes, sir.
The first plenary session on the morning of Wednesday, March 4th -- which is held on the morning of Wednesday, March 4th, was on the subject of evaluation criteria, correct?
A. Yes.
Q. Was the preamble presented and discussed at that session?
A. Yes.
Q. Who --
A. And it was presented on March 3rd, as well.
Q. All right. Who was the speaker or speakers at that session?
MS. WAGSTAFF: Same objection.

A. Dr. Straif.
BY MR. GRIFFIS:
Q. Dr. Kurt Straif?
A. Yes.
Q. And was he the only speaker?
A. As I recall, yes.
Q. What did Dr. Straif tell you about the criteria that you were to employ in evaluating the substances?
A. If it is in the preamble.
Q. So he told you that the methodology that should be applied during your review was what was set forth in the preamble, sir?
A. Yes.
Q. The next two plenary sessions, the mornings of the 5th and 6th were called progress report.
What happened at the progress report plenary sessions? I don't mean tell me everything anyone said. But, in general, what was the point of the progress report meeting?
A. A brief report on the previous day's meetings amongst subgroups.
Q. Did the subgroup chairs present at those meetings?

A. In general, yes.
Q. Okay.
A. It was the subgroup chair --
Q. Did anyone else --
A. -- present --
Q. Sorry.
A. I don't recall anyone else presenting.
Q. And what would the subgroup chairs -- what sort of thing would they report on? Let's just confine ourselves to mechanism.
What would Dr. Rusyn report on to the other groups?
A. So if --
MS. WAGSTAFF: Objection. Calls for speculation.
A. He would report on, in terms of the ten key characteristics, which of those ten might have evidence that would be considered strong, moderate, or weak.
BY MR. GRIFFIS:
Q. You were at all of these sessions, right?
A. Yes.
Q. Okay. The evening of Friday, March 6th, there was a plenary session called overview

discussion.
What was that about?
A. Plenary session overview was before the group as a -- as the plenary session, it was the -- it was the general overview of the evaluations of each compound. We had not met to go through the document line by line at that point.
Q. The two progress reports that we just talked about on the morning of the 5th and 6th were scheduled to be ten minutes long.
Were those, in fact, short meetings?
A. Yes.
Q. And then the evening session, the overview discussion was an hour and 45 minutes, right?
A. Yes, roughly. I don't remember the exact time.
Q. Okay. Now, while you were in Lyon, you were taking notes about the proceedings on the spiral bound notebook, and you produced some of those. Produced, again, meaning you turned them over to your lawyers, and they did what they did with them in response to request No. 3, right --

or Exhibit No. 3?
A. Yes.
Q. Okay. You had a spiral notebook, and you would take notes by hand as to what was happening that struck your interest.
Is that fair?
A. I don't -- the term "strike my interest," I -- that's not relevant.
Q. Okay. Well, you would choose what to write down and what not to write down, like anyone does who's taking notes is all I meant.
A. Yes.
Q. Okay. Exhibit 11.
(Exhibit No. 13-11 marked for identification.)
BY MR. GRIFFIS:
Q. What I've marked as Exhibit 11 is from your spiral notebook, and these are notes from the evening session on March 6th; is that right? Titled "plenary general remarks"?
A. Yes.
Q. Okay. Now, this notebook --
MS. WAGSTAFF: Objection. Those are from the evening session. There was two plenary sessions on March 6th.

BY MR. GRIFFIS:
Q. The morning session was ten minutes long, and the evening session was much longer. Which one was this?
MS. WAGSTAFF: If you know.
A. I don't recall if it was from the morning or the evening.
BY MR. GRIFFIS:
Q. Okay. We have four pages of notes, right?
A. I don't recall which one it was from.
Q. Okay. This is from one of the plenary meetings of March 6th?
A. It's from March 6th. That's my...
Q. I'd like to talk about the notebook for a minute. Was this notebook only -- and these questions are about the process that you went through to respond to our request in document No. 3, the subpoena for production of documents. Was this notebook devoted only to working group 112, or is it also a notebook that you used for other purposes?
A. It -- it was my -- it was a general notebook.
Q. So if we look back in February you might

have been writing about something you were doing in your lab or some other meeting that you went to; is that right?
A. Yes. You might have seen lab -- lab data that I had been working on.
Q. You --
A. Unrelated to volume 112.
Q. Sure. As one way of organizing your life, you keep a notebook keeping track of what you did and observed on various days?
A. Yes.
Q. Okay. So you pulled out the relevant notebook for when we provided you with that document request, Exhibit 3. You pulled out the relevant notebook and had copied the pages that pertained to working group 112; is that right?
A. Yes.
Q. Were there any notes from working group 112 that you didn't have copied?
A. I provided everything that I had regarding volume 112.
Q. You provided those to your lawyers?
A. Yes.
Q. Okay. And do you know whether they applied any selection process in deciding what to

send or not?
MR. WHITE: Only to your knowledge.
BY MR. GRIFFIS:
Q. Yeah. I am just asking if you know.
A. No. I don't know.
Q. Okay. And now let's go through your notes here, sir. Group 1, exposure. Group 1 was the exposure group, right?
A. Yes.
Q. Who was presenting as the head of group 1?
A. In this regard, these progress reports are general remarks that would have been the subgroup chair.
Q. Do you remember who that was?
A. For exposure, I'd have to look at the participant list.
Q. Okay. We have it. It's Exhibit 8.
MS. WAGSTAFF: Exhibit 8 is the assignment list.
MR. GRIFFIS: Yeah. The assignments is the closest we have to one with group 1 on it.

BY MR. GRIFFIS:
Q. Does the assignment list help you with that?
A. I think the list of participants says who the subgroup chairs are.
Q. Okay. The list of participants that we had from you was just for working group 4.
A. Let me just find -- which exhibit?
Q. Exhibit 8 is the one I was talking about, the one with the blue and white -- I see it here.
A. Oh, this one.
Q. No. There.
A. Oh, this one. Okay.
Q. Just see if that helps you remember who the chair was.
A. Trying to remember. I don't recall the group 1 subchair.
Q. Okay. That's fine, sir. The group 1 chair, whoever that was, was reporting on exposure assessment as a yes/no process, correct?
MS. WAGSTAFF: Object to the form.
A. They -- yes or no? I don't know what you -- can you rephrase that?

BY MR. GRIFFIS:
Q. Well, you wrote yes/no.
What did you mean?
A. I don't recall what I meant there.
Q. Okay. And you mentioned the Agricultural Health Study.
What point was made at this plenary session about the Agricultural Health Study with prior exposure assessment?
A. I don't recall. I don't know what compound this is -- this is relates to, which of the compounds.
Q. If you'll see, sir, on the first two pages were devoted to what looked like general comments. And then the next two pages were talking about specifics of various compounds. You have compounds listed over and over again on the last two pages and compounds generally not broken out at the bottom of Page 1 early on.
So do you recall from this session being given, first, an overview of the processes that each group was going through and assessing the data and then some specific findings?
A. They were giving overviews at their evaluations of their drafts. I don't remember

specifics.
Q. The undergroup 2, which is epidemiology, do you recall that being headed by Aaron Blair?
A. Dr. Blair was the chair of the whole committee.
Q. Okay.
A. Of the whole group.
Q. Do you know Dr. Blair?
A. I had met him one other time as a -- as a member of the Ag Health Study. He was an emeritus faculty at NCI. I had met him one time before the Lyon meeting.
Q. Okay. And CI.
What is CI?
A. National Cancer Institute.
Q. NCI. Okay. Thank you.
So I saw on Page 1 of your notes from the March 6th plenary session, sir. And it mentions -- says group 2, epidemiology, and then Agricultural Health Study. And then there's a list of exposure assessments below for TCPBP. There's parathion, malathion, and glyphosate.
Are those the exposure assessments from the Agricultural Health Study?
A. No.

Q. What are they from?
A. Those -- those -- these five compounds. Those -- that doesn't relate to the Agricultural Health Study.
Q. What does it relate to?
A. I believe these were the preliminary evaluations of the epidemiology group.
Q. As to glyphosate, it says, "Limited for NHL and inadequate for multiple myeloma;" is that right?
A. That's right.
Q. Okay. Now, if you turn over to the section on group 3, animal studies, do you recall who was presenting for that?
A. The group -- the animal subgroup was led -- the subgroup chair was Dr. Jameson.
Q. Did you have interactions with the other subgroups other than sitting in on the plenary sessions?
A. We interacted at coffee breaks, yes.
Q. Okay. And I mean, other than rubbing shoulders socially, did you have substantive scientific interactions with the other subgroups?
MS. WAGSTAFF: Object to the form.
A. I was not involved in subgroup 3 or

subgroup 2 or subgroup 1 to any significant extent.
BY MR. GRIFFIS:
Q. Okay. So you didn't have any substantive scientific interactions with members of those other subgroups as part of working group 112.
Is that fair?
MS. WAGSTAFF: Object to the form.
A. My main responsibility was to evaluate the toxicokinetic data for the five compounds that were charged.
BY MR. GRIFFIS:
Q. Okay. So is the answer, no, you didn't have substantive scientific interaction with the other three groups?
MS. WAGSTAFF: Same objection.
A. I wouldn't call it -- we didn't have substantive talks. We had discussions. I would -- substantive. I don't know. I can't characterize. That's hard for me to characterize.
BY MR. GRIFFIS:
Q. And I don't know if this is the thing that's getting you tangled up, but I'm talking about as part of an analysis of carcinogenicity of

these five substances, what you were all there for.

Rather than talking scientist to scientist about something of mutual interest; that wasn't what you were there for, right?

MS. WAGSTAFF: Object to the form.

A. So I did not have substantive discussion with the group 3 scientists regarding the cancer bioassay data on glyphosate. My charge was toxicokinetics.

BY MR. GRIFFIS:

Q. And did you have substantive interactions with group 1 or group 2 with regard to the carcinogenicity of glyphosate or the issues they were evaluating with regard to glyphosate?

A. Not that it impacted any of the evaluations.

Q. Okay. Do you know if Dr. Rusyn had substantive interactions with other groups, particularly with group 3?

MS. WAGSTAFF: Objection. Speculation. How would he know what Dr. Rusyn did?

A. I can't recall.

BY MR. GRIFFIS:

Q. Did Dr. Rusyn talk about having such

interactions?

MS. WAGSTAFF: Same objection.

A. I can't recall him...

BY MR. GRIFFIS:

Q. When your group met each day, did Dr. Rusyn report on what had happened the evening before during the closed coordination meetings for the co-chairs and subgroup chairs?

A. Perhaps in general terms, but I -- I can't remember specifics.

Q. Okay. Do you know if Kurt Straif was present at those coordination meetings?

A. I can't speak for these coordination meetings. These are the evening coordination meetings between the subgroup chairs --

Q. Yes.

A. -- and the overall chair of the meeting?

I can't speak because I wasn't present at those -- at those meetings.

Q. You didn't hear from Dr. Rusyn or anyone else about who was present or who was leading those meetings?

A. I presume Dr. Straif was there. But I -- again, I assume he was --

MS. WAGSTAFF: Objection.

A. Yeah.

BY MR. GRIFFIS:

Q. Okay. You would presume so, but you don't know?

A. I wasn't at the meeting.

Q. Yes, sir.

Under group 4, on the second page of your notes, sir, Exhibit 11, it says, "group 4," and then you wrote, "ten key characteristics of agents that cause cancer," correct?

A. Sorry. You're on page -- which page?

Q. Second page.

A. The second page. Okay. Ten key characteristics of agents -- yes.

Q. So this would have been a -- part of a presentation by Dr. Rusyn?

MS. WAGSTAFF: Objection. Foundation.

A. Yes.

BY MR. GRIFFIS:

Q. Okay. And the ten key characteristics of agents that cause cancer this is what you alluded to earlier as the ten bins into which you were to sort and analyze the mechanism of the evidence part of your methodology, right?

A. Correct.

Q. Okay. And now on the top of the third page, you again start listing group 1, group 2, group 3, group 4. And it appears that you've -- you're talking about the evidence that was presented as to parathion from 1, 2, 3, and 4, correct?

A. Yes.

Q. And then malathion?

A. Correct.

Q. And then diazinon?

A. Diazinon. Where is dizainon?

Q. The top of the next page.

A. Top of Page 4? Okay. Diazinon, yeah. Okay.

Q. Okay. And then towards the bottom of that page, you started talking about glyphosate, right?

A. Yes.

Q. Okay. Now, tetrachlorvinphos, was -- did you take notes on that and just not provide them to us, or not -- or what do you know?

A. There's something on TCBP. There's -- on Page 2, there's some -- I have some notes on TCBP.

Q. But not broken down by the four groups

like for the other substances, right?
A. No.
Q. Okay. Let's talk about the glyphosate notes on Page 4. Group 1. The report from group 1 share on glyphosate was -- that you wrote down was "detectable in water and food," correct?
A. Yes.
Q. Okay. For group 2, the report was glyphosate negative non-Hodgkin's lymphoma. Case control, glyphosate, arrow, non-Hodgkin's lymphoma, right?
MS. WAGSTAFF: Object to the form.
A. This -- this is what I wrote.
BY MR. GRIFFIS:
Q. And what's your recollection of what that meant?
A. I don't recall.
Q. Okay. And you also wrote AHS negative data, correct?
A. I did.
Q. And it is your understanding that AHS data was negative with regard to association with glyphosate?
MS. WAGSTAFF: Object to the form.
A. That is correct.

BY MR. GRIFFIS:
Q. And that is your understanding?
A. The AHS study. The AHS study, that was a negative result.
Q. Talking -- when you say the AHS study a negative result regarding glyphosate, are you talking about the DeRoos 2005 publication?
A. No. No. No. No.
Q. Tell me what you --
A. At AHS, there was a negative association, but there was a case control study that showed a positive association.
Q. Which study is that, if you recall?
A. I don't recall the citation.
Q. Okay.
A. But it's in the monograph.
Q. Yes, sir. Group 3. You wrote as your report from -- you wrote down from the group 3 report, "glyphosate limited to inadequate," correct?
A. Yes.
Q. Okay. So was it the finding of the group 3 group at that time that the evidence of carcinogenicity of glyphosate was limited to inadequate in animal studies?

MS. WAGSTAFF: Object to the form.
A. So I don't recall the specific discussion at this stage. This was early preliminary discussions. The meeting was only halfway through. So this was just a preliminary note in a plenary session.
BY MR. GRIFFIS:
Q. Yes, sir. Halfway through the group 3 -- group 3 had found limited to inadequate evidence of carcinogenicity of glyphosate, correct?
MS. WAGSTAFF: Object to form. There's no foundation that that's what group 3 actually found at that point.
A. I wasn't on group 3, so I wasn't privy to their discussions.
BY MR. GRIFFIS:
Q. That was reported to everybody at the plenary session; is that right?
A. I don't remember --
MS. WAGSTAFF: Objection.
A. -- the context, but this is what I wrote.
BY MR. GRIFFIS:
Q. Well, you participated in this, and you

attended multiple plenary sessions where you got progress reports.
Your understanding, halfway through, was that group 3 was trending towards limited to inadequate, as far as the animal studies point; is that correct?
MS. WAGSTAFF: Object to form and foundation.
A. They were only halfway through. They had not completed their evaluation. We hadn't even gone through the monograph as a whole -- as a -- in plenary session line by line. So I don't I -- I don't know which way they were trending at this point.
BY MR. GRIFFIS:
Q. What you wrote down from their report was "limited to inadequate," right?
A. That's what I have written down.
Q. And that would have been them, not you, because were not involved with group 3, as you just said?
A. My main focus was on the toxicokinetics in group 4.
Q. You didn't get involved with any evaluation of the animal studies.

Is that fair or not?
MS. WAGSTAFF: Objection to the word "involved."
A. I was not in subgroup 3 -- in their subgroup 3 discussions regarding the carcinogenicity of glyphosate in animals.
BY MR. GRIFFIS:
Q. Well, was the carcinogenicity of glyphosate in whole animals discussed in group 4?
A. I don't recall specifically. I don't recall whether the animal cancer bioassay data was discussed explicitly in our subgroup.
Q. Was human evidence -- by humans, I mean whole humans -- discussed in your group?
A. It wasn't in our subgroup.
MS. WAGSTAFF: Object to the form.
BY MR. GRIFFIS:
Q. I'm sorry. I didn't hear your answer.
A. We were focused on mechanisms. I was -- as a subgroup, we were focused on mechanisms. I was focused on toxicokinetics.
Q. For group 4 -- I'm going back to Exhibit 11 here, sir. For group 4, you just wrote glyphosate.
Do you recall what was being

reported as to group 4's findings at that point?
A. I don't recall.
Q. Okay. And can you tell the jury, since you were involved in all of these subgroup sessions for group 4, how group 4's thinking evolved over the course of work group 112?
MS. WAGSTAFF: Object to the form.
A. On which compound? On --
BY MR. GRIFFIS:
Q. Glyphosate.
A. Glyphosate?
Q. Yes, sir.
A. Okay. So the group was leaning towards looking at the data on the genotoxicity and oxidative stress of glyphosate and in evaluating that particular data. Because we concluded at the end -- by the end, we had concluded that the evidence was strong for those two key characteristics.
Q. Yes, sir. Over the -- over time, how did you evolve to the point of concluding there was strong as to those two characteristics?
A. I wouldn't use the word "evolve." I think the evidence was presented early on in the meeting that it was strong. I don't think there

was an evolution in that thinking.
Q. Okay. Were you always -- was your group always leaning towards the 2-A finding?
MS. WAGSTAFF: Object to the form.
A. Say that again one more time.
BY MR. GRIFFIS:
Q. Yes. The ultimate evaluation of IARC was to classify glyphosate as 2-A, correct?
A. That was the ultimate finding, yeah.
Q. And was that always group 4's view, or did that change over time?
MS. WAGSTAFF: Object to the form.
A. That was not always group 4's view, no.
BY MR. GRIFFIS:
Q. Tell me how --
A. Because we --
Q. -- group 4 changed over time.
A. Well, we don't make those evaluations in subgroup, like group 2-A or 2-B. Those are not made within the subgroup. Those are made as a whole, as a -- within plenary. Taking into account the human data -- the human epi data, the animal cancer bioassay data, and the mechanistic data. So evaluations are not made within individual subgroups.

Q. So your -- please correct me if I'm wrong.
But your task, as part of subgroup 4, the subgroup 4 task was to make an evaluation within the ten key cancer characteristics -- the ten bins that we talked about earlier as to weak, limited, or strong?
A. Correct.
Q. Okay. And then that would go to the group as a whole to see what to do with that information.
Is that fair?
A. We would give descriptors to the evidence regarding these to ten key characteristics and summarize that, and it would be presented to the preliminary group.
Q. And your conclusion -- I mean the conclusion you would present would be weak, limited, or strong as to each of those bins with rationale, of course, correct?
A. Which is in the monograph.
Q. Yes, sir. But am I correct that would be the evaluation?
A. Right. And that was -- that would be in the -- very clearly stated in the monograph, as it

was.
Q. And where is it written, if anywhere, how IARC evaluates the significance of a finding of strong for genotox and strong for oxidative stress?
A. Where is it -- explain what you mean.
Q. Yes, sir. Do you have some guidance for whether different substances are going to -- if evaluated in terms of the ten key characteristics of cancer, are different profiles, when divided among the key characteristics of cancer, right?
A. Yes.
Q. There are certainly substances for, example, for oxidated stress that show oxidative stress that aren't in fact carcinogens, right?
A. There are examples.
Q. And there are substances that are carcinogens that don't show oxidative stress?
A. But we're not talking about glyphosate here?
Q. No. No.
A. You are -- maybe this is hypotheticals now.
Q. It's true, though, correct?
MS. WAGSTAFF: Object as a hypothetical

and agree with the witness.
MR. WHITE: That's true. I've instructed my client not to answer any hypotheticals.
BY MR. GRIFFIS:
Q. Sir, when you were working with group 112, did you have any set of criteria by which you were to evaluate whether a substance was capable of causing human cancers based on the finding of strong or oxidated stress and strong for genotox?
A. We were instructed to evaluate the publicly available literature as a whole to determine whether there was strong evidence, moderate evidence, or weak evidence that glyphosate may cause oxidated stress or glyphosate may induce genotoxicity.
So we were instructed to look at the whole -- to the whole database and to draw conclusions whether the database was strong, moderate, or weak.
Q. When you say the whole database, you are referring to published literature and not to any industry studies that were conducted in GLP labs, correct?
MS. WAGSTAFF: Object to the form.

Suggestion that no industry studies that were conducted in GLP labs were part of the published literature?
A. We had access to the publicly available literature. It is my understanding that there were some industry studies that EPA had that we could get access to.
BY MR. GRIFFIS:
Q. Did you get access to them?
A. This for -- talking about the cancer bioassay data, they had access to EPA data.
Q. Do you know of any -- I'm going to use the term "registration study."
Do you know what that means?
A. For EPA. For data provided by the company to EPA for registration purposes.
Q. Did you look at any registration studies in reaching your evaluation about the mechanism?
A. I don't recall.
MS. WAGSTAFF: Object to the form.
A. There's -- I don't recall. The person who was looking at the genotox data may have, but there was data that was unavailable to the working group that Monsanto had access to.

BY MR. GRIFFIS:
Q. Do you know that there were publications presenting a great deal of that data, that Hyer & Kirkland published an article that was not reviewed by IARC?
A. And the reason was the committee couldn't evaluate the methodology that those studies used. They just presented a summary of findings without publishing the methodology involved. So independent scientists would have a very difficult time of determining the veracity of that data.
Q. And do you know what the methodological gaps that were listed in -- I mean in the IARC monograph, it says, we didn't look at the Hyer & Kirkland data because we couldn't evaluate A, B, C, D about the methodology.
Could you evaluate A, B, C, and D from all of the studies you did review from the published literature methodology fully set forth in those study?
A. For the -- I can only speak for the toxicokinetic data because that is what I was responsible for.
Q. Okay. You can't say as the genotox or

oxidated stress?
MS. WAGSTAFF: Objection asked and answered. He has given his response.
A. For the genotox and oxidated stress because I did not write those drafts. So I didn't look at every single one of those papers.
Q. Yes, sir.
A. I don't know -- I assume the -- for a paper to be brought forward and, especially if it was deemed to be a strong paper in terms of providing evidence for a mechanism, the -- you would need to see the methodology that was utilized in the statistical analysis and so forth.
So I'm -- I can't speak to that. I can't speak directly to that because I was not involved in the draft of that document, but this is publicly available literature. And it would be important for the reviewers for the -- for the committee to have that methodological information to evaluate the paper.
Q. Do you know who made the decision not to use the Hyer & Kirkland information?
A. I don't know who specifically was responsible for doing that.
Q. Who did you learn -- from whom did you

learn that that decision had been made?
A. I believe that it was -- it came up in plenary. And I don't remember if it was Dr. Straif or Dr. Guyton who determined that.
Q. Your belief is that it was either Dr. Straif or Dr. Guyton who rejected the Hyer & Kirkland data?
MS. WAGSTAFF: Object to the form.
A. Yeah. The specialist in the subgroup who worked on the genotoxicity would have been involved in that decision, as well.
BY MR. GRIFFIS:
Q. Okay. And do you know that, or is that just speculation?
A. I don't know for sure, but that's -- I assume the person who had -- who was in charge of that area would have been involved in discussions regarding that review paper, the cure paper.
Q. Who was that?
A. Who was the genotox specialist?
Q. Yes, sir.
A. On our subgroup?
Q. Yes, sir?
A. Dr. LeCurieux.
MS. WAGSTAFF: I am going to object to

this line of questioning. He's -- the deponent has said he doesn't know the answer. And he's also used the word that he's assuming. So I'm going to object for speculation.
MR. WHITE: And I'd like to add that you don't have to make any assumptions.
MR. GRIFFIS: What time is it?
MR. WHITE: 11:41.
MR. GRIFFIS: So we've been going an hour.
VIDEOGRAPHER: 44 minutes.
(Exhibit No. 13-12 marked for identification.)
BY MR. GRIFFIS:
Q. Okay. Dr. Ross, I handed you a document that you provided to us. It is an e-mail exchange between you and Dr. Michael Alavanja.
Is that pronounced correctly?
A. Yes.
Q. Okay. And would you please tell us who Dr. Alavanja is?
A. He was the principal investigator of the Agricultural Health Study at the National Cancer Institute.

Q. In this thread, he announced that he was retiring from NCI, correct?
A. Yes.
Q. Okay. You sent him your best wishes and then talked a little bit about AHS and the IARC meeting, correct?
A. Right.
Q. Okay. And do you know him through your role on the AHS, the advisory committee?
A. Correct.
Q. Is that the only way you know him, or did you have a prior relationship, as well?
A. Not before that.
Q. Okay. And you told him indeed the AHS worked out a prominent role at the IARC meeting I attended, right?
A. Yes.
Q. What did you mean by that?
A. Many of their studies were being evaluated at the meeting.
Q. And was it your understanding, from attending the plenary sessions and hearing the epidemiology group and exposure group talk about the Agricultural Health Study data, that it was important to their evaluation?

MS. WAGSTAFF: Objection. Dr. Ross stated he didn't -- wasn't involved in those subgroups. And, also, the Agricultural Health study involves other chemical besides glyphosate, which is outside the scope.

BY MR. GRIFFIS:

Q. Go ahead, sir.

A. The AHS studies was not just on glyphosate. There were other chemicals being evaluated, some of which were the organophosphates at the volume 112 meeting. So there was -- this is what I mean by AHS had a prominent role at the meeting.

Q. When you said a prominent role, you weren't talking about glyphosate? You were talking about the other substances?

MS. WAGSTAFF: Objection. Misstates the testimony.

A. I was talking about in general.

BY MR. GRIFFIS:

Q. Okay.

A. The AHS work in general.

Q. Did it have a prominent role with regard to glyphosate?

A. Well, it -- its data was evaluated in

the glyphosate -- in the evaluation of glyphosate. That study was evaluated.

Q. The whole group met to put all of this together, put the whole evaluation together to talk about all of the data, right?

A. The whole -- the whole group, yes. Sure.

Q. Yes. And was it your understanding from those meetings the AHS data was important to the evaluations of the glyphosate by the other groups?

MS. WAGSTAFF: Objection.

A. I wasn't in group 2.

BY MR. GRIFFIS:

Q. Talking about the meetings.

Everybody had to go together?

A. I can't recall that.

Q. You were at glyphosate issue -- back to Exhibit 12 and your e-mail to Dr. Alavanja.

"The glyphosate issue kind of blew up after we had finished and left," correct? What did you mean by it kind of blew up?

A. There was a lot of press.

Q. Then you said, "Although, it was the rodent cancer bioassays, in the case of glyphosate that was really the most controversial issue for

glyphosate," right?

A. That's what I've written.

Q. What did you mean?

A. There was debate going on within the cancer bioassay subgroup regarding whether it was deemed to be sufficient or limited. So there was debate -- scientific debate at the meeting --

Q. You --

A. -- regarding those -- that issue.

Q. You considered that to be the most controversial debate that was going on that you were aware of with regard to glyphosate at IARC 112?

A. Yes.

Q. Okay. And it was between limited or sufficient with regard to cancer bioassays for animals?

A. Yeah. I -- yes. It was -- it is that issue.

Q. And did you know who was advocating for limited and who was advocating for sufficient?

A. I don't remember. I can't recall.

Q. Okay. Do you recall anyone who was advocating for limited or sufficient?

A. No.

Q. Okay.

A. I wasn't privy to their conversations.

Q. Okay. Now, as a member of the AHS advisory group, are you made aware of the content of the data that hasn't been published?

MS. WAGSTAFF: Objection.

BY MR. GRIFFIS:

Q. That data they continue to collect hasn't been published?

MS. WAGSTAFF: His role as an AHS advisory member is outside of the requested discovery of the exploration of the mechanism subgroup's conclusion about glyphosate.

A. I don't receive any unpublished data from AHS.

BY MR. GRIFFIS:

Q. Do you receive -- you were giving them advice about things, right? Did they ever ask you whether you think something should be published?

A. No.

Q. What sorts of things did they ask for advice about?

A. We -- I have only met with them one time. They would ask studies -- they would ask opinion -- you know, ask us our opinion. And in

my case, they would ask my opinion about issues of measuring pesticide, residues, and issues of mechanistic mechanisms by which chemicals might cause cancer, mutations in cancer.

Q. Did you have an understanding, from your review of the preamble, your attendance at the evaluation criteria meeting, all the training you got on IARC methodology, that if the epidemiology evidence, evidence of group 2 is below limited, then the substance in question gets a group 3 classification?

MS. WAGSTAFF: Objection. Calls for speculation. Foundation.

BY MR. GRIFFIS:

Q. Do you recall that?

A. So if -- yeah -- wait a minute. The human epi, if it was deemed to be inadequate, and the animal cancer bioassay data -- well, it's -- we are speculating now because that is not what happened.

Q. Well, let's take a look at the preamble, Page 23.

You reviewed and understood the preamble, correct?

MS. WAGSTAFF: I'm actually going to

object also, this is causing for a hypothetical that is completely unrelated to the mechanism subgroup conclusion about glyphosate. You're actually proposing a hypothetical on what happens if the epidemiology has a different classifications as to what it ultimately determined.

MR. GRIFFIS: Well, I will link it up. Don't worry.

BY MR. GRIFFIS:

Q. Page 23.

A. Uh-huh (affirmative response).

Q. You see, the criteria for an evaluation of group 3, "This category is used most commonly for agents for which the evidence of carcinogenicity is inadequate in humans and inadequate or limited in experimental animals," right?

A. Correct.

Q. Okay.

MS. WAGSTAFF: I'm going to object to you're saying that that is a "shall make" determination.

MR. GRIFFIS: Let me finish, please.

BY MR. GRIFFIS:

Q. "And, exceptionally, agents for which the evidence of carcinogenicity is inadequate in humans but sufficient in experimental animals may be placed in this category when there's strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans," right?

A. That's what the preamble says.

Q. In group 4, "This category is used for agents for which there is evidence suggesting lack of carcinogenicity in humans and in experimental animals," right?

A. Yes.

MS. WAGSTAFF: Continue to object on the scope, as it seems as you're trying to elicit expert testimony.

BY MR. GRIFFIS:

Q. Sir, did you know that Dr. Aaron Blair was deposed in this litigation?

A. Yes.

Q. Did you talk to Dr. Blair about being deposed?

A. No.

Q. Do you know about that fact that he was

deposed?

A. I found it in the court records.

Q. Did a little research when you heard you were going to be deposed?

A. We are scientists. It is publicly available.

Q. Did you know Dr. Blair disclosed that the AHS has seven more years of follow-up data than that that was presented to IARC and that that data, which involves many more cases than has been previously published in DeRoos in 2005, the article that was considered by IARC, is strongly negative for non-Hodgkin's lymphoma and that if that data had been put into the meta analysis and was done by the epidemiology group, the relative risk would have been below 1.0. About 0.9.

Did you know that?

MS. WAGSTAFF: Objection. Misstates the -- Dr. Blair's testimony and is completely irrelevant. And you're doing a hypothetical upon hypothetical.

MR. WHITE: You can answer as to whether or not you were aware that that was...

A. No. I wasn't aware of that.

BY MR. GRIFFIS:
Q. Okay. Do you know what relevance the findings of the mechanism group would have in the presence of negative human epidemiology in the absence of a limited association?
MS. WAGSTAFF: Objection. Calls for a hypothetical. If it was presented in this particular monograph 112, then that is appropriate, but I think you're exploring hypotheticals that are inappropriate to the scope.
BY MR. GRIFFIS:
Q. Go ahead, sir.
MR. WHITE: You can answer as far as you have factual knowledge of a yes or no, but you do not need to go into any details of a hypothetical.
A. The mechanistic subgroup can upgrade or downgrade if -- if it needs to. So I -- since that wasn't the issue in this case, then, I don't know what else I can add.
BY MR. GRIFFIS:
Q. Well, this is a question about the -- your understanding of the methodology applied by IARC in doing its classifications and how

mechanism fits into that. What --
A. But then I have to go into a hypothetical.
Q. What is the role of mechanism in the absence -- in the presence of negative human epidemiology? Negative, not limited.
MS. WAGSTAFF: Objection. Hypothetical.
THE WITNESS: So should I answer this hypothetical?
MR. WHITE: You can answer it to the extent that you -- that you know under this evaluation, under the way that you were instructed.
A. Right. So if it was inadequate in humans, sufficient in animal, and we had strong evidence in mechanism -- mechanistic evidence, then we could call for an upgrade to upgrade the classification.
BY MR. GRIFFIS:
Q. To 2-A?
A. If it was inadequate -- yes. Look at -- you can look in the preamble. Okay.
Q. Show where it shows the inadequate evidence in human --
A. Page 22, line 35. "In some cases, an

agent may be classified in this category, being 2-A, when there is inadequate evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals and strong evidence that carcinogenesis was mediated by a mechanism that also operates in humans."
Q. What strong evidence was presented in the IARC monograph working group 112 that carcinogenesis observed in experimental animals is mediated by a mechanism that also operates in humans?
MS. WAGSTAFF: Objection to the monograph. It speaks for itself.
A. The mechanistic evidence that was deemed strong was the genotoxicity and the oxidative stress classification. You know, just those characteristics.
BY MR. GRIFFIS:
Q. So just the fact of finding genotoxicity and oxidative stress suffices to show this is a mechanism that operates in humans.
Do you have to be more specific than that?
A. Because the findings, the data, were obtained in exposed humans in cultured cells -- in

vitro human cells -- cultured in vitro, exposed to glyphosate. And in some animal models, in vivo there was evidence of carcinogenicity -- or excuse me. Take that back -- of genotoxicity.
The important thing, in terms of operable in humans, is the fact that exposed humans showed evidence of genotoxicity, and cultured cells of human origin showed evidence of genotoxicity. Those were -- those then showed that this mechanism may operate in humans.
Q. You would agree with me that genotoxicity does not mean carcinogenicity, right?
MS. WAGSTAFF: Object to the form.
A. As -- not all genotoxins lead to cancer.
BY MR. GRIFFIS:
Q. And that is because there are multiple additional steps that have to take place before cancer is produced, right?
A. Yes.
Q. Geno toxicity would have to lead to a permanent mutation in order to cause cancer, correct?
MR. WHITE: I'm going to object. At this point, we're moving beyond the scope of IARC, and we're asking for expert testimony.

You don't have to answer that.
BY MR. GRIFFIS:
Q. Sir, in order to reach a conclusion that the genotoxic mechanisms that you identified as part of working group 112 can operate in humans, there would need to also be evidence that those genotoxic mechanisms would lead to permanent mutations, not just temporary, transient ones, correct?
A. The evidence would be stronger if it was permanent mutations.
Q. If there was evidence -- if, in fact, the evidence was not consistent with permanent mutations, than the genotoxic mechanism that you observed couldn't produce cancer in that way, correct?
MS. WAGSTAFF: Objection. Calls for a hypothetical.
A. I don't know. I can't say anything to that. I don't know.
BY MR. GRIFFIS:
Q. That wasn't part of your evaluation?
A. Well, if it leads to DNA damage, this could lead to genomic instability and cancer. So just to rule out DNA damage is not causing -- DNA

damage can lead to mutations.
Q. And DNA damage might not lead to mutations, as well?
A. It depends on the context.
Q. There are all sorts of analyses and assays that are done to look for actual mutations such as AIMS test, right?
A. There are.
Q. Okay. And that evidence is negative for glyphosate?
A. It is in the monograph. Whatever the AIMS assay showed, it's in the monograph, whether it was positive or negative.
Q. You don't know?
A. I think for the AIMS assay, the data for glyphosate is negative.
Q. Yes, sir.
MR. GRIFFIS: We'll break now then for lunch?
VIDEOGRAPHER: Off record at 11:59.
(A lunch recess was taken.)
VIDEOGRAPHER: Back on record. This is DVD three at 1:05.
(Exhibit No. 13-13 marked for identification.)

MS. WAGSTAFF: Just for completeness of record, we had the phone line open all day, and we don't believe anyone has called in; and no one has made a peep.
BY MR. GRIFFIS:
Q. Dr. Ross, I hand you Exhibit 13. And that is an e-mail from Dr. Rusyn to you at Martin and Frank LeCurieux -- did I pronounce that right?
A. Correct.
Q. Dated February 27th of 2015, correct?
A. I am just looking for the actual e-mail here. Let's see. Which page is it? Is it -- from -- that's from Kate Guyton and Ivan.
MS. WAGSTAFF: I'm just going to put an objection on the record that there is a document that was produced or provided by Dr. Ross. It is a more complete cascade of this conversation. And the fact that it's not to all of those folks. It's just to Dr. Guyton.
BY MR. GRIFFIS:
Q. You see the top of this document?
A. I got cc'd on it.
Q. Okay. And Dr. Rusyn responded to Kathryn Guyton and cc'd you and suggested that you

take a look at some of the subjections that were attached to that document, right?
A. Yes.
Q. And the document in question was the Greim published article; is that correct? Greim 2015?
A. I am not familiar with that article. I think -- is this the article with the -- there were several studies summarized?
Q. Yes, sir. A summary of multiple animal studies. Greim, et al., 2015.
A. Okay.
Q. And Dr. Rusyn forwarded that to you with the suggestion that you take a look at the small vignettes that are relevant to your subsection on mechanistic data; is that correct?
A. Yes.
Q. Dr. Rusyn said, "With regard to the Greim article, this is an interesting prelimical piece," correct?
A. Yes.
Q. And did you view the Greim article as a prelimical piece?
A. I didn't have an opinion on it.
Q. He said -- Dr. Rusyn said, "It does not

surprise me that, when under pressure, the industry can muster a relevant publication." He put relevant in quotes. "It goes from submission to acceptance in as little as seven weeks," correct?

A. That's what is written there.

Q. Okay. And what did you understand him to mean by the industry being under pressure?

MS. WAGSTAFF: Objection. Calls for speculation.

A. I didn't know what he -- I didn't know what he meant by that.

BY MR. GRIFFIS:

Q. Now, you worked with Dr. Rusyn closely during working group 112 and got to know him and his style of working, right?

A. I got to know Dr. Rusyn.

Q. Okay. And is his sarcastic tone towards industry consistent with your experience working with him on working group 112?

MS. WAGSTAFF: Object to the form. There's nowhere on here that it says it's sarcastic.

A. I didn't find him sarcastic. I found him objective.

BY MR. GRIFFIS:

Q. Did you find this paragraph -- "This is an interesting prelimical piece. It does not surprise me that, when under pressure, the industry can muster a 'relevant' publication. It goes from submission to acceptance in as little as seven weeks. Kudos to CR-2, a known helper to 'informative' publications from the industry stakeholders for such expediency and relevancy."

You don't find that to be sarcastic?

MS. WAGSTAFF: Objection. If you want to know if it's sarcastic, you need to ask the person who wrote it and not someone who is merely cc'd on the document. This is beyond the scope of -- of the subgroup's determination on glyphosate.

A. I don't have an opinion.

BY MR. GRIFFIS:

Q. Did Dr. Rusyn express any views about industry to you during working group 112?

A. No.

Q. Did he express any views to you about whether he felt that the chemicals that you were investigating should be more strongly regulated

than they were during working group 112?

A. No.

Q. Okay. He said at the end of his e-mail, "I am confident that the IARC monograph will be much more comprehensive and balanced," correct?

A. Yes. That's written here.

Q. And the IARC monograph did not include the Greim article or the studies discussed therein, correct?

A. Right.

Q. Did not discuss the Hyer & Kirkland article or the studies discussed therein, correct?

A. Correct.

Q. Okay. Now, you're aware, because of the correspondence that you were a signatory to following IARC, that there are a number of regulatory agencies that have also done reviews of glyphosate both before and after the IARC review; is that right?

MS. WAGSTAFF: Objection. This is completely beyond the scope. Anything that happened after IARC is not allowed by the scope of the order allowed by Judge Charbrio and MDL.

A. So -- okay. Is your question did I know

of anything before the meeting?

BY MR. GRIFFIS:

Q. No, sir. Question is, because you were a signatory to some letters, following IARC, you are aware that regulatory agencies have also done reviews of glyphosate, both before and after working group 112 met?

MS. WAGSTAFF: Objection. Again, this is completely beyond the scope of what is allowed by this deposition. The regulatories -- decisions have nothing to do with the mechanism subgroup's conclusion of glyphosate, especially when you're talking about after monograph 112.

A. So I was not aware of EFSA doing their regulatory review until after it came to light --

BY MR. GRIFFIS:

Q. Yes, sir.

A. -- that I understood what was going on there. So I am aware that regulatory agencies have been reviewing glyphosate, yes.

Q. And are you -- and you're aware, because it's part of the substance of the letters that you signed, that those reviews involved a review both of the published literature and the unpublished,

right?
MS. WAGSTAFF: Again, this is completely beyond the scope of what's allowed, and this is an abuse of the order that Judge Charbrio entered allowing exploration of the mechanism subgroup's conclusion about glyphosate. You're asking about letters that happened after monograph 112, and you're asking about regulatory agencies which haven't even been allowed in this litigation.
MR. WHITE: Yeah. At this point, I'm going to instruct my client that he does not have to answer these. It's not -- if it's not brought back to the actual monogram.
MR. GRIFFIS: I'm bringing it back.
MS. WAGSTAFF: I think he was instructed that he didn't have to answer it.
BY MR. GRIFFIS:
Q. Do you know that Dr. Jameson testified today that he wasn't shown the Greim article -- Dr. Jameson?
MS. WAGSTAFF: Objection. We don't have any authority or any foundation that that's true. And we have no idea what the testimony question was asked or what was said. That's

pure speculation. How would he know that?
MR. WHITE: You don't have to answer that.
BY MR. GRIFFIS:
Q. Do you know if Dr. Jameson was shown Greim?
MS. WAGSTAFF: Objection. Speculation.
MR. GRIFFIS: Okay. I'm going to mark another document.
(Exhibit No. 13-14 marked for identification.)
(Exhibit No. 13-15 marked for identification.)
MS. WAGSTAFF: Did you highlight these, Kirby, or is it --
MR. GRIFFIS: This is how we have it.
MS. WAGSTAFF: Okay. Wait.
MR. WHITE: We have two -- 14 and 15?
MR. GRIFFIS: Yes, sir.
MS. WAGSTAFF: Which one do you want as 14?
MR. GRIFFIS: 14 is that one.
BY MR. GRIFFIS:
Q. This is from the documents that you provided to us, sir. Okay. Marked as Exhibit 14

is some comments by Chris Portier on a response by EFSA to a letter sent by Portier and others.
And 15 I marked because it's the -- it has numbered paragraphs also supplied by you. Numbered paragraphs that link up to the numbered paragraphs in Mr. Portier's --
MS. WAGSTAFF: I'm again going to object. The request for this deposition was to explore the mechanism subgroup's conclusions about glyphosate. And that is what the Court allowed as a fact deposition. And now you are asking about something that happened in January 13th, 2016, which is a year and a half after the conclusion came out. And I think it's a completely inappropriate line of questioning.
MR. GRIFFIS: It links directly to the procedures used by IARC at the group.
BY MR. GRIFFIS:
Q. I just want to ask you about one comment by Chris Portier, sir.
This is a document that you recognize that came from your production, right?
MS. WAGSTAFF: You're talking about Exhibit 14?

MR. GRIFFIS: Yes.
MS. WAGSTAFF: Okay. I object as to foundation. This is from Chris Portier. Nothing on here that shows him as the author.
BY MR. GRIFFIS:
Q. Sir, first of all, do you recognize this as a document that you were sent?
A. I mean, I can't recall, but if -- you know, if this was under the subpoena...
Q. It's a document that you provided to us. I will tell you that.
A. If that's the case then, yes, then I -- then I would say, yeah, it was swept up. But I don't recall this specifically.
Q. Okay.
MS. WAGSTAFF: I object to any questions on this document as the deponent said he doesn't recall it.
BY MR. GRIFFIS:
Q. Do you recall Mr. Portier communicating with you about the responses that he was putting together in asking you to be part of it and sign responding to EFSA?
A. Yeah. We -- I was one of a approximately 93 people.

Q. Yes, sir. And it says, "Thoughts on EFSA response. See EFSA response."

Are these Chris Portier's thoughts or your thoughts?

MS. WAGSTAFF: Object to any questions on this document as the deponent has stated he doesn't remember this document.

A. These are not my comments.

BY MR. GRIFFIS:

Q. Okay. Comment on paragraph 19, "After carefully reading the current RAR, they may be correct" -- that's R-A-R -- "they may be correct in saying that IARC could have used these data. However, second guessing this at this time is wasted effort."

See that, sir?

MS. WAGSTAFF: Objection to asking questions on this document, as the deponent has said he does not recall it. He also stated these are not his comments.

BY MR. GRIFFIS:

Q. You see that, sir?

A. I see it. These are not my comments.

Q. No, sir. I'm not saying that they are. Chris Portier's comments.

Would you go to paragraph 19 in Exhibit 15 so that we can see what he's talking about?

MS. WAGSTAFF: Objection. No foundation. Chris Portier's comments.

A. Exhibit 15.

BY MR. GRIFFIS:

Q. Yes, sir. See these paragraphs are hand numbered, and they match up with the comments on the other. That's why I produced this one to you.

A. Okay. Paragraph 19?

Q. Right. And paragraph 19 reads, "I wish to make a final but important point regarding transparency. The background documents display detailed information on how EFSA and Member States appraised each study, including industry sponsored studies and how all those which participated, except Sweden, concluded that glyphosate is unlikely to pose a carcinogenic hazard to humans."

Did I read that correctly?

A. Yes.

Q. Okay. So my question to you now, sir, is, do you agree that IARC could have used those data that were reviewed by EFSA and not reviewed by IARC?

A. IARC -- the preamble -- sorry.

MS. WAGSTAFF: I was going to say an objection to using this document, as the deponent has said he does not recall this document, and this is calling for an expert -- calling for expert testimony and hypotheticals when he has stated all along that they followed the procedures as set forth in the preamble.

BY MR. GRIFFIS:

Q. So your answer?

A. The preamble asked us to look at the publicly available literature.

Q. Okay. Could IARC -- I don't mean -- was it a -- was it consistent with IARC's rules or would it have been against the rules or not -- as a scientist, doing a review of the science on the mechanism, could you have used the additional data found in the industry studies that were reviewed by EFSA and other regulators?

MS. WAGSTAFF: Objection. You're asking him whether or not he should have broke from IARC procedure, and I think that puts the deponent in a very uncomfortable position; and it's an inappropriate question.

BY MR. GRIFFIS:

Q. Let me be clear. I'm not asking you if it would have been good for you to go ahead and break with IARC procedures. I'm asking you, as a scientist, doing what's supposed to be an objective evaluation of the available evidence on glyphosate, would it have been useful to you to have even more evidence to look at, i.e., the evidence looked at by EFSA and not by IARC?

MS. WAGSTAFF: Object.

BY MR. GRIFFIS:

Q. Would that have improved or made worse your evaluation of mechanism?

MS. WAGSTAFF: Objection. Foundation. We don't even know what the data is you're talking about -- the strength, weaknesses the biases, anything with respect to that data.

MR. WHITE: When answering this, just answer to the best of your ability with -- from your own knowledge. All right? You don't need to speculate on whether or not you should or should not have been using data that was not provided to you.

A. I don't know the answer to your question. I don't know without -- I can't

speculate. I feel like I would be speculating.
BY MR. GRIFFIS:
Q. Because you don't know what that data shows?
A. The form of the data, where it's published, I would -- I think it's speculative for me to say.
Q. Based on your understanding of the methodology that you were to follow as part of working group 112, would more information that is negative weaken your conclusion of a strong association, or is that not the way the methodology works?
MS. WAGSTAFF: Objection. Calls for a hypothetical and speculation on what would have happened had some fictitious data been available pursuant to the preamble.
BY MR. GRIFFIS:
Q. Do you understand the question, sir?
A. I do.
Q. Okay. So now -- and what it is, is given the procedure that you're following, given the methodology that IARC asked you to follow, you had evidence of genotoxicity that you considered to be strong. You had evidence of oxidative

stress that you considered to be strong.
What does the methodology say you are to do with additional negative information about genotoxicity and additional negative information about oxidative stress? Would that weaken or have no effect on a conclusion of strong?
MS. WAGSTAFF: Objection. Calls for a hypothetical. Again, talking about data that is not allowed under the preamble.
MR. WHITE: I advise you to only answer to the extent that you know under the preamble. All right?
A. Preamble says we were to evaluate the publicly available literature, and that's what we did.
BY MR. GRIFFIS:
Q. Do you know, in working group 118 and working group 119, they looked at non-published literature?
MS. WAGSTAFF: Objection. This is completely outside the scope when we're talking about other monographs. We're here to talk about monograph 112 and specifically the mechanism subgroup. And now you're

bringing up monographs 117 and 120 that we know absolutely nothing about.
BY MR. GRIFFIS:
Q. 118 and 119. Did you know that, sir?
MR. WHITE: If we -- if this isn't going to be brought back to the monograph that's actually at issue, I'm going to instruct him not --
MR. GRIFFIS: It is, sir. It is.
BY MR. GRIFFIS:
Q. Do you know that IARC doesn't always follow what you're saying is the rule of only looking at published literature? Do you know that?
MS. WAGSTAFF: Completely beyond the scope of this deposition. I object for that.
MR. WHITE: You don't have to answer that.
BY MR. GRIFFIS:
Q. Sir, do you know why the leaders of IARC chose not to look at unpublished data in working group 112?
MR. WHITE: To the extent of your knowledge.
A. Because it wasn't in the publicly

available database.
BY MR. GRIFFIS:
Q. And do you know why they chose to look at unpublished literature in other monographs?
MS. WAGSTAFF: Objection. Foundation. And beyond the scope allowed by this deposition.
MR. WHITE: To the extent of your knowledge.
MS. WAGSTAFF: And calls for speculation. How is he supposed to know what other people did or didn't do?
A. I didn't know.
BY MR. GRIFFIS:
Q. Were you aware before today that IARC doesn't necessarily follow a rule of not looking at unpublished data?
MS. WAGSTAFF: Objection. Foundation. Timing and the scope of this deposition. And his attorney has already instructed him not to answer on that.
MR. WHITE: That's true. You don't have to answer that.
BY MR. GRIFFIS:
Q. Sir, you came to working group 112. You

followed the rules. The rules, as you understood them, didn't permit you to consider registration studies, didn't permit you to consider data generated by industry, and didn't permit to consider -- although you weren't part of the decision -- the Greim data or the Hyer & Kirkland data.

Is that all correct?

MS. WAGSTAFF: Objection to the phrasing of that whereas it was the rules as he considered it. Later monographs looked at unpublished data for one reason or another as you're apparently representing. We have no idea if the rules change. We have no idea under what circumstances that happened. And we have no idea of any facts surrounding that method. It's beyond the scope of the deposition.

MR. GRIFFIS: I object to the continued speaking deposition [sic] which are taking more transcript than my questions.

BY MR. GRIFFIS:

Q. Everything I just said is true, right?

A. We were instructed to evaluate the publicly available literature.

Q. Right. And you know that there was a body of registration studies, a body of industry studies. There were studies mentioned in the Greim article study. There were studies mentioned in Hyer & Kirkland. And you were not to consider any of those.

You did know that, right?

A. I didn't know the specifics of the industry studies.

Q. Okay. And you didn't look at those studies, I know, but you know that such studies existed and that you weren't going to be looking at them?

A. I didn't know the scope of the industry studies.

Q. Okay. Do you know today that there are such studies?

A. Based on the Greim article?

MS. WAGSTAFF: Scope.

BY MR. GRIFFIS:

Q. Based on the Greim article.

You were copied on that e-mail before you went to working group 112 attaching the Greim article, right?

A. Yes.

Q. Okay, sir. And is it fair to say that you don't know what your conclusions would have been with regard to mechanism had you seen those studies.

Is that fair?

A. I can't speculate on that because we didn't see it.

Q. Right. So you're agreeing with me.

You don't even know what -- you didn't know how that would have affected your analysis?

A. I can't speculate on that because we were instructed to look at the publicly available literature.

Q. Okay. Now, I am going to ask you a question about the methodology that you were asked to follow.

And this isn't about whether you look at publicly available literature or not. This isn't about that facet of the methodology prescribed to you by IARC. It's about a different facet.

My question is this, sir. Were you instructed, if you find multiple articles that show, in your view, a strong genotox signal and

multiple articles that show a strong oxidative stress signal, plus there are a whole bunch of other articles in those same categories that are negative, what are you to do with the negative articles? Do they tend to weaken your conclusion, as to strong association, or they have no impact on it because you already have a number of articles showing this association?

Do you understand my question?

A. So we look at the overall database, and we try to balance it with positive articles -- articles that suggest strong evidence versus negative evidence. So we are trying to look at the entire database as a whole and weigh that.

Q. So you were weighing the evidence. And if there was negative evidence that would tend to count against a conclusion -- a strong conclusion with regard to genotox or oxidative stress or any of the other ten cancer characteristics, right?

A. I believe the -- in the monograph that the tables lay out in a balanced way several of the positive studies and some of the negative studies, but on balance, there were more positives than negatives that helped us draw a conclusion.

Q. Right. And right now I'm not asking

about how those studies came out in your -- in your weighing. I'm asking you about what you understood to be the rules that you were following in doing the weighing. And I believe you're telling me your understanding was that, to the extent that there are negative studies in a particular category, those tend to count against a finding of strong.

And to the extent that there are positive studies, they tend to count for a finding of strong, and you -- you weigh them; is that correct?

A. Within the publicly available literature, we try to weigh both sets of data.

Q. Okay. And so you try to weigh both sets of data within the literature that you were provided as part of working group 112 and the publicly available literature that you found. And you -- and to the extent that there was negative data in that data set, it counted against your conclusion of strong.

That's fair?

A. We would weigh all the studies together, positive and negative.

Q. All right. Is your lab here at MSU a

GLP lab?

A. No.

Q. Are there any GLP labs at MSU?

MS. WAGSTAFF: Object to scope. Whether or not Mississippi State University has a GLP lab has nothing to do with the mechanisms of that group's conclusions about glyphosate, completely irrelevant.

MR. WHITE: You can answer to your knowledge?

A. I'm not aware. I don't know if there are or not.

BY MR. GRIFFIS:

Q. Okay. Do you know generally how GLP certification is achieved?

MS. WAGSTAFF: Objection. This is not relevant to the scope of this deposition.

MR. WHITE: Only to your knowledge.

A. My only knowledge is from work I did in a contract lab back in the early '90s that was GLP certified. So that is my knowledge of GLP.

BY MR. GRIFFIS:

Q. Okay.

A. When I worked in a contract lab.

Q. Okay. You worked in a GLP lab?

A. Yes.

Q. And your -- there were independent auditors in that lab, correct?

A. We would have auditors that came in either from the company or from government, in EPA, for example.

Q. The company auditors -- I don't know if you knew this or not -- but did you know that they were required to have a different management than the management of the lab so that they're reporting to different people?

MS. WAGSTAFF: Objection. This is getting way beyond monograph 112 and whether or not he knows about the management of GLP labs.

A. I don't know that level of detail about GLP.

BY MR. GRIFFIS:

Q. Okay, sir.

(Exhibit No. 13-16 marked for identification.)

BY MR. GRIFFIS:

Q. Sir, Exhibit 16 is an e-mail from you to Dr. Rusyn, March 11th of 2015, which is the day you left Lyon, right?

A. Yes.

Q. And you told him, "You did a fantastic job as chair," and asked to keep in touch, right?

A. Yes.

Q. Okay. And you were responding to a March 9th -- you weren't responding to the substance, but you clicked respond on a March 9th e-mail from Dr. Rusyn, correct?

A. Yes.

Q. Okay. And Dr. Rusyn wrote, "I would like to convene group 4 downstairs in the first coffee break to discuss the information below," correct?

A. Yes.

Q. Okay. And March 9th was the second to last day of working group 112, right?

A. Yes.

Q. Okay. This e-mail -- we don't have some of the header information. In Dr. Rusyn's e-mail, your system that you were using didn't include it.

But was this e-mail sent to you and the others in group 4?

A. I would -- it was sent to me. I would assume all the members received it.

Q. And did you, in fact, convene downstairs

in the first coffee break to discuss the information?
A. We did to discuss a potential upgrade.
Q. Okay. And what do you mean by upgrade?
A. The mechanistic upgrade. If animal data was considered limited and the human epi data was considered limited by the IARC rubric in the preamble, if there was mechanistic information that was considered strong by the subgroup, we could consider an upgrade.
Q. So you wanted to make sure we were all on the same page, we being group 4, correct?
A. Yes.
Q. Lower the evaluations from groups 2 and 3 in the IARC matrix. You apparently attached the matrix; although, that didn't come through in what you sent us, right?
A. Where's the matrix? I'm sorry. I don't see what.
Q. I'm reading from the e-mail. "Just to make sure we're on the same page, below are the evaluations from groups 2 and 3 and the IARC matrix."
A. Oh, okay.
Q. And there's some image that was attached

but didn't come through in what you provided to us, presumably the matrix.
"To get us to understand where our conclusions fit." That's what he wrote, right?
A. Yes.
Q. With regard to glyphosate, he said, "human limited." That's group 2, finding of limited. Group 3, finding of limited.
Correct?
A. At this -- well, at -- I don't know what was going on in group 2. I am not privy to their conversations, but it is -- it says "animal, limited" there. So he was convening a meeting --
Q. He says below --
A. -- to discuss --
Q. Yes, sir.
And he was -- this is at 9:00, so it's after both plenary sessions for the day, right?
MS. WAGSTAFF: Objection. Where do you see that it's at 9:00?
MR. GRIFFIS: I'm sorry. I'm wrong. It's at 4:42.
BY MR. GRIFFIS:
Q. It's at a break from the plenary

session, correct?
MS. WAGSTAFF: Well, object to that. We don't if it's a.m. or p.m.
A. I don't know what time it is.
BY MR. GRIFFIS:
Q. Were you taking a coffee break at 4:42 a.m. or 4:42 p.m., sir?
A. No. This was not a -- we were meeting -- the first coffee break, that would be in the morning.
Q. The first coffee -- so was this meeting to be held on the 9th or the 10th?
A. I don't recall.
Q. All right. Anyway, he was -- he said, "Below are the evaluations from groups 2 and 3." And the evaluation that he reported from group 2 was human glyphosate -- human, limited. And the evaluation that he reported for group 3 for glyphosate was animal, limited. Correct?
A. That's what's written here.
MS. WAGSTAFF: Object to the form.
BY MR. GRIFFIS:
Q. And what would -- you were in the plenary sessions, right, sir?
A. Yes.

Q. What was the basis for the finding of limited in the animal study group as of March 9th?
MS. WAGSTAFF: I'm going to object to the suggestion that these were announced at the plenary session. Nowhere on here that I can see does it say that Dr. Rusyn got this from the plenary session. We don't know where he got them from.
A. I don't recall what -- the discussion regarding the limited evidence.
BY MR. GRIFFIS:
Q. Do you know, sir, whether Dr. Rusyn got this from a public session that you were present at or from a closed session where only he and a few other people were present?
A. I don't know.
Q. Do you know where Dr. Rusyn got the impetus to ask for an upgrade?
MS. WAGSTAFF: Objection. Calls for speculation.
A. Part of the rubric or the preamble gives the mechanistic group the ability -- well, to propose an upgrade if the evidence warrants it.
BY MR. GRIFFIS:
Q. He says -- okay. And I want to finish

out my question.

Do you have any understanding as to the basis for the animal group's evaluation, as of March 9th, being limited?

MS. WAGSTAFF: Objection. Asked and answered.

A. I don't know. I don't know the basis of what was -- what they considered limited.

BY MR. GRIFFIS:

Q. Earlier you told -- you testified that, in your opinion, the most controversial issue with regarding to glyphosate was group 3's classification as between limited and sufficient with regard to particular animal tumor data; is that right?

A. This was the main issue. This was an important issue. There was a lot of debate about it.

Q. And when did you witness that debate or hear about that debate?

A. In the plenary session.

Q. There was debate at the plenary session between limited and sufficient in the animal study group; is that right?

A. There was -- in the early plenary

session, there was -- there was debate. There was further analysis going on, but I was not privy to all that data analysis because I am not a cancer biologist. So it was out of my -- my expertise.

Q. What was being said by the advocates for the limited view in those sessions that you witnessed advocating for a limited finding?

A. What was said?

Q. Yes, sir.

A. I don't recall.

Q. Who was making -- who was making the points in favor of a limited deal?

MS. WAGSTAFF: Objection. Asked and answered. He said he didn't know that.

A. I really don't recall who was arguing. At this stage, I was busy getting my drafts together, doing some fact-checking. I know there was lots of debate. It wasn't in my area of expertise, so the -- in the conversations that were going in the group 3 where I wasn't present for it.

Q. And in evaluating it as the most contentious issue with regard to glyphosate at working group 112, what were you basing that on? Hearing people argue and not understanding the

arguments or what?

A. No. There was a --

MS. WAGSTAFF: Objection. Argumentative.

A. Yeah. There was a lot of debate. There was a lot of scientific debate about the evidence about -- and how it fit with the preamble.

BY MR. GRIFFIS:

Q. And as you're sitting here, you can't remember anything about that debate or who was advocating on which side?

MS. WAGSTAFF: Objection. Asked and answered.

A. I -- I don't recall. I -- I don't recall the limited -- who was advocating for limited. I don't recall who -- who was advocating for a limited stance.

BY MR. GRIFFIS:

Q. Was it only the members of the -- of group 3 who were having that debate, or was Chris Portier or Kurt Straif or Dr. Rusyn or anyone else also participating in it?

A. There was debate with the whole group in the plenary session. There was debate going on with several scientists.

Q. Any from group 4?

A. Yes.

Q. Who?

A. Dr. Rusyn. He was -- he was debating the evidence.

Q. He was advocating for a finding of sufficient, correct?

A. I don't -- that word "advocate," I -- you know, I don't recall if it was -- he didn't use the word "advocate."

Q. Yes, sir. You used the word "debate" earlier.

A. Yeah. Debate about the evidence. Or there's debate about how to deal with this animal cancer bioassay data. We had, you know, multiple species getting tumors, different types of tumors, so there was debate there.

Q. What analyses or reanalyses of the cancer data are you aware of from being a participant in working group 112?

MS. WAGSTAFF: Objection. He testified he did not participate in the animal subgroups.

A. I don't know what analyses or reanalyses were being conducted. I know on the -- on the --

they have -- they stated in the monograph what statistical analyses were being used. But I am not familiar with what was done.
BY MR. GRIFFIS:
Q. Okay. Was Chris Portier involved in the debate over whether the animal group conclusion should be limited or sufficient?
A. I don't recall him specifically. I don't can't recall.
Q. Was Kurt Straif involved in that debate?
MS. WAGSTAFF: You now asked him seven different times if he recalls who was involved in the debate on which side, and every time he said he doesn't recall. So I'm not quite sure we need to stay on this topic.
A. I don't recall if Kurt was involved in the discussion. He may have been trying to form -- you know, mediate, be a moderator, as his role as the head of the IARC monographs. But that's, I mean, certainly not advocating for one side or the other.
BY MR. GRIFFIS:
Q. Dr. Rusyn says, after he reports that the animal group, as of March 9th, was -- had a finding of limited. "I have questions on the

limited in animals because there are two studies showing significant effect."
You see that, sir?
A. Yes.
Q. Did Dr. Rusyn express during this coffee break meeting or any other time his position that limited was the wrong conclusion and sufficient was the correct conclusion for the animal studies group?
MS. WAGSTAFF: Objection as to scope. This deposition was noticed to explore the mechanism subgroup's conclusions about glyphosate, and you are directly asking him about some other person's opinion on the animal subgroup.
A. I think he was questioning these two studies showing a significant effect, and I don't recall which two studies they are. Again, I don't think he was strongly advocating limited or sufficient at that time.
BY MR. GRIFFIS:
Q. During this coffee break meeting or at any other meetings with Dr. Rusyn, did he express in front of you what his questions were on the classification as limited?

MS. WAGSTAFF: Same objection as to scope. This deposition was noticed to explore the mechanism subgroup's conclusion about glyphosate, and you're asking him questions about some other scientist's opinion on the animal subgroup.
A. I don't recall what his questions were about limited.
BY MR. GRIFFIS:
Q. Again, sir, the point of this meeting -- this coffee break meeting on the second to last day of working group 112 was to talk about an upgrade, which is an interaction between the mechanism group's conclusions and those of the animals study's group to alter the classification; is this right?
MS. WAGSTAFF: Object to the form.
A. It was meeting to -- as to whether the mechanistic subgroup should bring forward to the whole group in the plenary session whether a mechanistic upgrade should be voted on or asked for.
BY MR. GRIFFIS:
Q. Tell us what happened at this meeting.
A. Which particular meeting?

Q. The first coffee break meeting that Dr. Rusyn convened on the second to last day of working group 112?
A. So it dealt with the mechanistic evidence we had. We had given the qualitative descriptor of strong to both the genotoxicity data and the oxidative stress data. These were two of the ten characteristics of the human carcinogens. And the debate or the question that was being raised was whether we bring it forward to upgrade -- as an upgrade in the plenary session. Was it -- was the group comfortable with that approach.
Q. Was Dr. Rusyn's recommendation that the group bring it forward, and he was seeing if you were comfortable with that approach?
MS. WAGSTAFF: Objection. Scope.
A. It wasn't his recommendation. He took a straw poll of the group -- of the subgroup.
BY MR. GRIFFIS:
Q. Did he lay out the analysis before he took the straw poll?
A. The analysis was in the monograph in the drafts of the mechanistic section. So the rationale is in the monograph for labeling the

genotoxicity data as strong evidence and the oxidative stress data as indicating strong evidence. So the rationale was there. So we were familiar with that.

Q. Okay. And as to all three of the substances that he wanted to talk about -- malathion, diazinon, and glyphosate -- he was either supporting saying we support the classification in 2-A or suggesting considering upgrade to 2-A, correct?

A. This is for glyphosate?

MS. WAGSTAFF: Object.

BY MR. GRIFFIS:

Q. For malathion, diazinon, and glyphosate.

Should I ask the question again, sir?

A. Let me just read this.

Q. Sure. Okay.

A. Okay, sir. Your question?

Q. Yes, sir. In this meeting that Dr. Rusyn convened on the last day -- second to last day of working group 112, with regard to all three of the substances that he addressed in his e-mail, you were either already at 2-A or he was suggesting considering an upgrade to 2-A; is that

right?

A. For malathion, we were at 2-A.

Q. And for the other two, he suggested considering an upgrade to 2-A, right?

A. He was -- yes. He was asking whether we should consider an upgrade to 2-A.

Q. And the group decided to upgrade to 2-A as to both of those, right?

A. Glyphosate, we didn't upgrade. Right. We did -- didn't -- there was no upgrade because the final conclusion for the human data with limited evidence -- and for the animal data, it was considered sufficient based on IARC's rubric, that constitutes a 2-A classification. So we did not need to propose an upgrade.

Q. Well, when you walked out of this meeting, what had you decided about proposing an upgrade?

A. That's while the meeting is going on. So we -- he had taken -- we had taken a straw poll, and we supported the proposal to upgrade if necessary. That never occurred, though. That never happened because it was 2-A based on the animal data and the human data.

Q. So the outcome of this coffee break

meeting on March 9th was the mechanism group agreeing to support an upgrade as to diazinon and to glyphosate, but it never became necessary for the mechanism group to put that into effect at a plenary session because the animal group moved; is that right?

A. For glyphosate.

Q. For glyphosate.

What happened with diazinon?

MS. WAGSTAFF: Objection. Scope. Irrelevant to this litigation.

A. I can't recall. We'll have to look at the monograph.

BY MR. GRIFFIS:

Q. Okay. Was Chris Portier at that meeting, coffee breaking?

A. I don't recall.

Q. Okay. And, sir, I have some questions for you about your understanding of the nature of the review that you were conducting as a member of working group 112. I'll show you a document on that first. Okay. If I can find it.

(Exhibit No. 13-17 marked for identification.)

MR. GRIFFIS: I only have two copies of

that.

BY MR. GRIFFIS:

Q. Okay. Sir, on March 30th of 2015, someone named Nathaniel Harmon, who I assume you didn't previously know, e-mailed you saying he worked for Guide Point, inviting you to talk to a client who was an institutional investor about glyphosate; is that right?

A. Yes.

Q. And you declined the invitation but told Mr. Harmon some things about the nature of the evaluation that you had performed as a member of working group 112; is that right?

A. Yes.

Q. First of all, you corrected him that it wasn't a study.

It was a review of scientific literature, right?

A. Yes.

Q. And you stress that IARC deals with hazard identification as opposed to a risk assessment; is that right?

A. Correct.

Q. And hazard identification, as you described to Mr. Harmon, is a classification

indicating the strength of the evidence that a substance can cause cancer, right?
A. Correct.
Q. And it's different than a risk assessment, which defines the level of carcinogenic risk for individuals; is that right?
A. Correct.
Q. And you referred him to the IARC preamble on that subject?
A. Yes.
Q. Okay. And you have the preamble there, sir. The preamble is Exhibit 10.
A. Okay.
Q. On Page 2, sir, the preamble in the third full paragraph under objective and scope --
A. I'm sorry. What page?
Q. Page 2.
A. Page 2.
Q. Under the heading of objective and scope.
A. I'm not finding it.
Q. The pages -- when I say Page 2, I mean the page numbered 2, not the second page.
A. Can you point it out to me?
Q. I'm sorry. The numbers start here.

A. Okay. Got you.
Q. There's no numbers on the first two pages. Page 2, objective and scope, third full paragraph. This is -- this is the methodology that you were following. "Cancer hazard is an agent that is capable of causing cancer under some circumstances; while a cancer risk is an estimate of the carcinogenic effects expected from exposure to a cancer hazard," correct?
A. Yes.
Q. Okay.
A. That's what the IARC preamble says.
Q. And it says -- it goes on to say in that same paragraph that, "The monograph identified cancer hazards even when risks are very low at current exposure levels, and that's because new uses or unforeseen exposures could engender risks that are significantly higher; is that right?
A. Yes.
Q. Okay. So under this hazard versus risk approach, it is possible for a substance to be a hazard without actually being a risk to causing human cancers.
Is that fair?
MS. WAGSTAFF: Objection. Calls for

expert opinion. And it's -- you've just asked him to admit that the IARC doesn't look at risk assessments, so now you're -- you shouldn't be asking about risk assessments as a fact witness on the IARC 112.
A. This -- so your question is hazard -- hazard versus risk?
BY MR. GRIFFIS:
Q. Yes, sir.
A. And we were dealing with a hazard assessment in IARC. Risk assessments was not our job.
Q. Right. And I just wanted to -- these questions are so that we can understand and the jury can understand what you understood yourself to be doing as a member of working group 112. That's why I'm asking you about this, sir.
You understood, as a member of working group 112, in identifying glyphosate as being a cancer hazard, that it could be that humans would not be exposed to glyphosate at a level that could be a threat to them, whether it's a hazard or not. True?
MS. WAGSTAFF: Objections. Calls for expert opinion. He's now said two times that

he didn't do risk assessments. So asking him whether or not humans are exposed at a level that's dangerous is a back door way of asking for an expert opinion, and it's inappropriate.
A. I'm not an expert in risk assessment. My role here was to study the toxicokinetic database.
BY MR. GRIFFIS:
Q. And you were a member of the whole working group on the entire issue of mechanism, right?
A. Correct.
Q. Okay. Based on your work and your conclusions and what the mechanism group did, the mechanism group's conclusions do not translate to a statement that glyphosate is capable of causing cancer in humans at levels at which humans are actually exposed.
Because you didn't look at the exposure issue, correct?
MS. WAGSTAFF: Objection. Calls for expert opinion. It's not a negative or a positive finding in that way, I believe that the doctor has said.

A. There is an exposure subgroup in the IARC panel that deals with exposures.
BY MR. GRIFFIS:
Q. No. The --
A. So there is evidence of exposure, human exposure.
Q. Yes. Whether humans are exposed.
A. Right.
Q. And there's some information as to the ways that they're exposed.
But my question is a little different, sir. As a member of working group 112 and a member of the mechanism subgroup, your conclusions about glyphosate being a hazard with regard to carcinogenicity does not translate into a statement that glyphosate is capable of causing cancer in any particular actual human at the levels to which they are exposed?
MS. WAGSTAFF: Objection. Calls for an expert opinion. That's not what he's tested, and he's has admitted he's not an expert on risk assessment. This line of questioning is inappropriate.
MR. WHITE: I believe he's answered more than one time that the analysis that they did

was for -- not for risks but for hazards. I'm not sure that we need to keep asking the same question.
BY MR. GRIFFIS:
Q. Okay. So that the jury can understand what you understood yourself to be doing and the meaning of the procedure you were following in following the preamble, sir, it is true that we can't conclude that any particular human being ever got cancer from glyphosate from IARC's findings.
Is that true?
MS. WAGSTAFF: Objection. Calls for expert opinion. Misstates the testimony and the preamble.
MR. WHITE: Yeah. You only have to answer to the extent of your knowledge based on hazard versus risk. You do not have to offer any kind of opinion.
A. I think you're asking me to give an opinion.
BY MR. GRIFFIS:
Q. I'm asking you to help the jury understand what hazard means, that you were doing a hazard assessment and that you were aiming to

point out the difference between hazard and risk, which you told them is done by regulatory bodies -- risk assessment if done by regulatory bodies.
MS. WAGSTAFF: I object. You're asking him to take the hazard definition and the risk definition as put in the preamble and apply the risk definition to what they -- the IARC found about hazards. And I feel that that is an expert opinion, and I feel that his attorney is appropriate in instructing him not to answer.
BY MR. GRIFFIS:
Q. IARC did not find that any human ever got cancer from glyphosate, right?
MS. WAGSTAFF: Objection. Misstates the record.
A. IARC's conclusion is that glyphosate falls under two way designation. Probably carcinogenic to humans. And that's, I think, all I can say.
BY MR. GRIFFIS:
Q. Is it consistent or inconsistent with a finding of 2-A, given the scope of the review that you conducted and given that it was a hazard

assessment, that glyphosate has never caused cancer in any human being?
MS. WAGSTAFF: Objection. You're calling for an expert opinion again. He's just told you that all he can say is that glyphosate -- or that IARC found it a 2-A. And now you're asking him to apply and come up with an expert opinion, which is inappropriate.
A. I'm not an expert in risk assessment, so I can't really give you an answer on that.
BY MR. GRIFFIS:
Q. Okay. Sir, so is it fair to say that you can't say whether IARC's conclusion that glyphosate is classified as 2-A is consistent with glyphosate never having caused any actual human cancer?
MS. WAGSTAFF: Objection. You're doing a back door question to get him to give an expert opinion, and that's inappropriate.
BY MR. GRIFFIS:
Q. You can't say?
MS. WAGSTAFF: Same objection. Calling for expert opinion. I think it's inappropriate.

MR. WHITE: You can answer whether or not you have knowledge but not --
A. Glyphosate was deemed to be 2-A by the working group.
BY MR. GRIFFIS:
Q. Yes, sir. And as a member of the working group, I just wanted to know whether it's your understanding that glyphosate could be 2-A and that no human being ever got cancer from glyphosate. Because that's a risk issue, not a hazard issue.
Is that your understanding, or am I wrong about that?
MS. WAGSTAFF: Objection. Once again, you're calling for an expert opinion. He's told you what IARC did as a hazard report. He told you the conclusion. And you're asking him to apply a risk assessment.
A. I can't say for sure -- you don't know. You don't -- 100 percent certainty that glyphosate never caused cancer, you can't say that.
BY MR. GRIFFIS:
Q. You can't say one way or the other?
MS. WAGSTAFF: Objection. Calls for an expert opinion.

MR. WHITE: You don't have to answer that. We've been down this. You've asked the same question a number of times, and he's given his answer.
MR. GRIFFIS: Let's take five minutes.
VIDEOGRAPHER: Off record at 2:04.
(A short recess was taken.)
(Exhibit No. 13-18 marked for identification.)
VIDEOGRAPHER: Back on record at 2:11.
BY MR. GRIFFIS:
Q. Doctor, I handed you Exhibit 18, which is an Environmental Health Perspective, and I believe this is one you alluded to earlier in the deposition, correct?
A. Yes.
Q. This is the document setting forth what you've called a few times the 10 key characteristics of carcinogens; is that right?
A. Yes.
MS. WAGSTAFF: Objection. Misstates the testimony. He stated they were on the website. And I object to any documents that were after IARC being within the scope of this deposition.

BY MR. GRIFFIS:
Q. Okay. Sir, where did you -- how did you come to understand that the source of the 10 key characteristics of carcinogens which you were to apply as a member of working group 112 came from the Environmental Health Perspective document?
A. Well, Kate Guyton, the meeting rapitor, was an author on it. So she was aware of this article. This was received 5th of March. So she was aware, and she had given us a Powerpoint presentation on these key characteristics as a way to prepare for evaluating the data. There was a -- I believe it was on the IARC website, too.
Q. So Kathryn Guyton had you follow this procedure as part of your methodology. And it was submitted -- it was received by the journal actually during the working group's review; is that right?
A. Yes. It was received.
Q. And it's correct that it hadn't been accepted for publication until after working group 112 had already left; is that right?
A. Yes.
MS. WAGSTAFF: Object to the question. He stated that these 10 points were on the

IARC website unrelated to a publication that they were a policy of the IARC. So any suggestion that this was unpublished manuscript we would object to.
BY MR. GRIFFIS:
Q. Do you know, sir, if the procedure that you followed of putting carcinogens into ten different bins was a published peer-reviewed procedure before working group 112?
A. So this -- this paper -- the idea of characteristics of carcinogens actually derives from an earlier paper published in Cell about the 10 different cellular mechanisms that can happen during the carcinogenic process and cancer progression.
So it was -- there was a Cell paper published -- oh, a few years ago by some eminent cell cancer biologist who -- who brought up the issues that these key characteristics of carcinogens might fit into, like cell proliferation, receptor mediated effects genotoxicity, DNA repair.
These -- these known mechanisms by which a cell becomes a cancer cell, the various steps that have to take place.

Q. And did these Cell articles propose using those the ten characteristics as a screening tool for hazard?
A. No. No, not at all.
Q. Do you know --
A. This is -- yeah -- no.
Q. Okay. So this is the first publication that proposes using those ten characteristics as a screening tool for hazard?
A. This one right here, DHP article, the mechanistic data is vast, so this was a way to organize and consolidate and compile the data --
Q. Okay. So as a --
A. -- in a logical way.
Q. Yes, sir.
So as a methodology, this process that you went through, this methodology that you applied as a member of working group 112, didn't get published and peer reviewed until after you had already left Lyon.
Fair?
A. This article wasn't in -- yeah. In press until after the -- until after the meeting.
Q. Okay. I'd like to take a look at the authors, sir.

A. Uh-huh (affirmative response).
Q. And, first of all, have you heard of either the Ramazzini Institute or the Collegium Ramazzini?
A. No.
Q. Never been asked to be a Ramazzini fellow?
A. No.
Q. Okay. And do you know of any link between the Ramazzini Institute or the Collegium Ramazzini and IARC?
A. No.
Q. You ever heard of a Ramazzini fellow?
A. No.
Q. Okay. And I don't know well, sir. You're making a face and shaking your head.
A. Oh, I'm sorry. This Ramazzini.
Q. Does it ring a little bell, or you just have no idea what --
A. No. I'm sorry.
MS. WAGSTAFF: Are you seeing that word on here, or is that just a different question?
MR. GRIFFIS: It's not on here.
MS. WAGSTAFF: Okay.

BY MR. GRIFFIS:
Q. Do you know, sir, that multiple authors of this paper and multiple signatories of EFSA letter that you were asked to sign off on and the differences letter that Chris Portier asked you to sign off on were members of the Ramazzini Institute or the Collegium Ramazzini?
A. No.
Q. Okay. You don't know anything about the funding of the Ramazzini Institute or Collegium Ramazzini?
A. No.
Q. Okay. This -- in this paper under the acknowledgment section on Page 2, it says, "We thank all other members of the 2012 working group who attended the workshops in Lyon, France," and, of course, you weren't part of a working group in 2012; is that right?
A. Thank all members of the 2012 working group?
Q. Yes.
A. Did you say volume 12?
Q. 2012.
A. 2012 working group. Yeah. Yeah. I wasn't a member of that.

Q. All right. And on Page 4 in the Smith article, sir, under background, the second sentence, it says, "This exercise was complicated by the absence of a broadly accepted systematic method for evaluating mechanistic data to support conclusions regarding human hazard from exposure to carcinogens."
Did I read that right?
A. Yes.
Q. Okay. Is it correct that, as of the time the working group met, there was not a broadly accepted systematic method to evaluate mechanistic data to support conclusions about human hazard to exposure to carcinogens?
A. I think there were approaches to consolidate the data, but this was an attempt to logically place the evidence in these -- in these 10 key characteristics.
Q. And since this article was submitted for publication, have there been other attempts by others authors to do that?
A. I believe IARC uses this as their approach in all -- all mechanistic evaluations now.
Q. Yes, sir. I'm asking something

different. I'm asking about published literature on the subjective use of mechanism in hazard assessment.

Has anyone else proposed an alternative methodology to this one?

A. Not that I'm aware of.

Q. Okay. Is that an area of literature that you follow -- that you'd be likely to know or just don't happen to know?

A. It's not -- no. I just don't know.

Q. Okay. Now, on Page 6, I'm looking at the middle paragraph and starting about the middle of it.

"Herein, we describe" -- you see that?

A. Uh-huh (affirmative response).

Q. "Herein, we describe these 10 key characteristics and discuss their importance in carcinogenesis. These characteristics are properties that human carcinogens commonly show and can encompass many different types of mechanistic influence. They are not mechanisms in and of themselves, nor are they adverse outcome pathways."

Did I read that right?

A. Yes.

Q. Could you explain to the jury, please, what it means -- the statement that "they are not mechanisms in and of themselves" means and what the statement "they are not adverse outcome pathways" means?

MS. WAGSTAFF: I'm going to object to the use of this document as it was clearly developed and finalized after the monograph 112, and Dr. Ross was not an author of this document. And he has testified that he -- that they have a similar set of 10 characteristics, but not this document.

A. I don't really follow -- I mean, I'm not sure what is meant by this sentence, as I didn't write this sentence. I believe adverse outcome pathways relates to risk assessments.

MS. WAGSTAFF: Objection. Calls for speculation on what others meant.

BY MR. GRIFFIS:

Q. This material -- I mean, this is Kathryn Guyton's proposal for how hazard assessments should be done, and she presented on this to you, correct?

A. This is of this whole group here, but

Dr. Guyton did present to us the key characteristics -- the 10 key characteristics.

Q. And that's the procedure you followed?

A. And that is.

Q. Okay. You don't understand what was meant by, "These 10 key characteristics are not mechanisms in and of themselves"?

A. I'm not -- I'm clear on what this is meant -- "they are not mechanisms in and of themselves." I am not -- I can't read the mind of the author.

Q. Let's go to Page 10. Characteristic 2 is genotoxic, and this is one of the two of the ten characteristics where the working group 112 found a strong connection, correct?

A. Correct.

Q. The weight of the evidence that you evaluated was strong, right?

A. Correct.

Q. I am looking at the first full paragraph under genotoxic and the last sentence, "DNA damage by itself is not a mutation," correct?

MS. WAGSTAFF: Are you asking if that's what it says, or are you asking --

MR. GRIFFIS: So far I'm asking if

that's what it says.

A. Yes.

BY MR. GRIFFIS:

Q. Okay. And it is true, right? DNA damage is not a mutation?

MS. WAGSTAFF: Object to the form.

A. DNA damage is -- can lead to a mutation.

BY MR. GRIFFIS:

Q. And in order for DNA damage to lead to cancer, it needs to cause a mutation, and that mutation has to be one that affects the cell in a way that leads to unchecked proliferation of cells, correct?

MS. WAGSTAFF: Objection. This is calling for expert testimony and not the mechanism subgroup's about glyphosate.

A. So my direct responsibility was to do the toxicokinetic evaluation.

BY MR. GRIFFIS:

Q. Yes, sir. And let me ask you about that. There are -- in the IARC monograph, there are multiple sections, correct? And multiple sections that the working group -- that your group, group 4, was responsible for collectively, right?

A. Yes. So my section was specifically toxicokinetics. I wasn't writing on any of the 10 key characteristics in terms of draft form.
Q. Yes, sir.
A. I wasn't responsible for that.
Q. So if we went through in detail the IARC monograph and looked at -- I mean, for example, there's a section that addresses genotoxicity, right?
A. Uh-huh (affirmative response).
Q. And it has multiple studies -- multiple tables, and those tables list multiple studies, and there are summaries of what the study showed or didn't show.
All of that is in there?
A. Correct.
Q. Would you be an appropriate person to ask about the significance of those tables and the evaluation of those tables and what it said in those studies and the significance of those studies to a finding of genotoxicity or not?
A. I have a background in DNA adduct research as a graduate student and as a post doc. So I -- yes. There are aspects that I would be appropriate too -- it would be appropriate for me

to evaluate as a group -- as a mechanism subgroup.
Q. And let me be clear. I wasn't asking whether you'd be qualified to review those studies. I'm sure you would.
My question is whether, as you sit here today, based on the knowledge in your head and the work that you did in working group 112, you would be qualified to answer detailed questions about those studies, about the tables, about the significance of the studies to working group 112's evaluation of genotoxicity?
A. Well, it's -- it's -- it was a long time ago. Now, I am familiar with the evaluation, and it's in the monograph.
Q. Okay.
A. So I -- uh-huh (affirmative response).
Q. Okay. Well, I asked the questions about the layout of the monograph and your expertise because you said, look, I was in charge of pharmacokinetic sections. So would you explain to us the distinction between the pharmacokinetics section which you wrote in the first instance and -- I'll wait for your mic to go back.
Okay. Would you explain to us the distinction that you were trying to make between

the pharmacokinetic section, which you wrote in the first instance, and the other sections of group 4 in terms of what you know and can testify to and give opinions about?
A. Right. So I wrote the drafts on the toxicokinetics, the drafts that were started six months before the meeting. That was my main responsibility. I was at the meeting as this evidence is being presented, the genotoxicity evidence and the oxidative stress evidence.
And as a peer reviewer, as a scientist peer reviewer, we are asked to evaluate those studies and decide whether they are strong evidence, moderate, or weak evidence. So we are peer reviewing in that process the data that's being presented and the arguments that are being presented.
Q. For example, with regard to glyphosate and the multiple studies that were cited in tables 4.1, 4.2, 4.3. 4.4, 4.5 of the monograph and subject to genotoxicity, did you read all those studies?
A. I did not.
Q. Okay. Did you read many of those studies?

A. We had points -- you know, there were leads on each of those sections -- on genotoxicity, for example --
Q. Yes, sir.
A. -- who were responsible for evaluating those studies and writing summaries about what that data meant.
Q. Sure. And they presumably read them all, but you did not?
A. Yes. We did not have time.
Q. Okay. And you didn't have time because you weren't just looking at genotoxicity. You were looking other bins, and you were looking at four other chemicals?
A. There was a lot of data.
Q. Correct.
On the oxidative stress section, that's where you did a peer review before you came, and you testified that you spent about a day and a half of total work on the peer review, including writing up the comment, which took a day.
Did you read all of those studies?
A. Some of the studies where I wanted to understand the method that was used to measure

oxidative stress, I looked at those papers.
Q. So you pulled some of the papers to look up the methodology --
A. I was interested in that.
Q. -- in those papers, and, otherwise, you didn't read the oxidative stress studies unless cited?
A. I did not read every single study that was cited.
Q. Did you read many of the oxidative stress studies in entirety?
A. I can't put a number on it.
Q. Okay. As to the other characteristics, the other 10 characteristics -- and I won't list them all here -- did you read the studies cited by working group 112?
A. For the other -- for receptor mediated and so forth?
Q. Receptor mediated, et cetera?
A. Those studies -- those characteristics weren't considered strong, so less -- less weight was put on them.
Q. It's even less likely that you would have read them; is that right?
A. Yes.

MS. WAGSTAFF: Object to form.
BY MR. GRIFFIS:
Q. Okay. On Page 20, sir. Well, first of all, let's go to Page 18. And the Smith article has a header here on Page 18. "Using the key characteristics to systematically identify, organize, and summarize mechanisms of information." Then there's a step one and on subsequent pages, step two and step three. And this is the methodology that was presented to you by Kathryn Guyton that the working group followed?
MS. WAGSTAFF: Object to the form.
A. I don't know if she presented it in exact same detail as here.
BY MR. GRIFFIS:
Q. Do you want to take a minute to read three steps and see if this is the procedure that you followed?
A. So one issue is I wasn't binning the -- I wasn't tagging this information for glyphosate. I mean, the toxicokinetics --
Q. I'm sorry. When I say the procedure you followed, I meant working group 112, not you personally as to every aspect of it.
A. In general, yes. We used we used HAWC

to tag studies. I think, in general, yeah, this is -- it's fair. To help us compile the relevant information.
Q. Under step 3, the first sentence is says, "It is increasingly evident" -- under step 3, the first sentence, "It is increasingly evident that multiple biological alterations or sets of different perturbations are necessary to convert a normal cell to a transformed cell and ultimately a tumor."
Did I read that right?
A. Correct.
MS. WAGSTAFF: Can you tell me where you're reading from?
MR. GRIFFIS: Yes, sir. Step 3 on Page 20?
MS. WAGSTAFF: Oh, first sentence.
MR. GRIFFIS: Yes, ma'am. First sentence.
BY MR. GRIFFIS:
Q. So a -- an insult, like a genotoxic insult causes DNA damage. More things need to happen in a cascade of events before that will produce a tumor and produce a cancer.
Is that fair?

MS. WAGSTAFF: Objection. Calls for expert opinion. This has nothing to do with how monograph -- a subgroup of the mechanism came to a conclusion of glyphosate, whether or not he believes that.
A. So I'm not a cancer biologist.
BY MR. GRIFFIS:
Q. Yes, sir.
A. It is out of my expertise, but there are several steps that have to take place. And that's cited by Hanahan & Weinberg. That was the article I was referring to. Multiple -- there's -- there are multiple steps in cancer.
Q. That's the article from Cell that you were referring to earlier?
A. Yeah. Yeah.
Q. Thank you.
Well, as someone who had -- who is on the mechanism subgroup, did you understand yourself to be trying to identify mechanisms by which glyphosate could actually produce cancer in human beings?
A. So the 10 key characteristics are what's known -- human carcinogens, human cancers that are formed by carcinogens like tobacco smoke, they

have usually two or more of these key characteristics. They go through a mechanisms that includes at least two or more of those key characteristics to cause tumors.

And so we were trying to use those key characteristics to evaluate the glyphosate database. We were trying to compile the data within those key characteristics to see where the strength of the evidence lay.

Q. And did you consider it to be part of what you were doing to figure out if the mechanisms you were looking at could actually induce that chain of events that could lead hypothetically to human cancer?

MS. WAGSTAFF: Objection. Your question just says hypothetically. And now you're again asking about the risk assessment and back-dooring an expert opinion. And I do not think this is an appropriate scope to ask about risk.

A. So it -- of course, if we could identify mechanisms, that would be important in any evaluation in terms of how a compound causes cancer.

BY MR. GRIFFIS:

Q. Yes, sir. Did you understand it to be -- from the briefings that you got about the methodology that you were to follow, the methodology set forth in the preamble, et cetera, that it was part of what you were there to do -- you being all of working group 112, not necessarily you personally -- to figure out how these mechanisms could actually lead to cancer in human beings or if they did?

MS. WAGSTAFF: Same objection.

A. We were charged with determining whether there was evidence in the glyphosate database -- the publicly available database that it had aspects of these 10 key characteristics, was -- what was the strength of evidence for those 10 key characteristics.

BY MR. GRIFFIS:

Q. And did group 4 take the next step of linking up what you found with regard to the 10 key characteristics, the two that were strong with regard to glyphosate to any additional steps in the chain between DNA insult and on one end of the chain and cancer on the other end of the chain?

A. So what we identified in subgroup 4 in

terms of genotoxicity was that the mechanism was operable in human cells. Mechanism -- the key characteristic of genotoxicity, actual damage to the nucleic acids. So that was deemed to be operable in humans and human cells in vitro.

Q. Yes, sir.

And did you also reach any conclusions about whether the mechanism then led to the next step in carcinogenesis or whether it may have stopped there?

A. We had strong evidence for genotoxicity and for oxidative stress.

Q. Okay. Do you understand what I'm asking you, sir?

A. I think I do, but I -- I don't --

Q. Okay.

A. I'm just telling you what we have.

Q. Yes, sir. I do. I understand what you have.

So you agree with me that there are potential insults to DNA on one side that would include oxidative stress and the genotoxicity findings that were set forth in the monograph. And then in order for actual human cancers to be created, there would need to be a series of

additional events, like mutations, for example. Like mutations.

And my question is, did the mechanism group or any other group you know of as part of working group 112 find any of those additional steps occurring -- find that the mechanisms actually produced any of the additional steps -- caused mutations, caused mutations that lasted, caused mutations that weren't repaired, caused mutations that were relevant to produce cancer, led to cancer?

MS. WAGSTAFF: Objection. You're asking the same question that the attorney -- that Attorney White told him not to respond to earlier, and that is an expert opinion on the risk assessment. And when you said probably 15 times, have you ever found that it caused it in humans, and he -- and right before the end. And now you've just rephrased your question, and you're asking it again. I think that's inappropriate, and I object.

BY MR. GRIFFIS:

Q. And to be clear, sir, what I'm asking you is whether IARC or whether the mechanism group or anyone else at IARC that you know of followed

the chain of evidence that you see and found any further than identifying the initial insult to DNA.

MS. WAGSTAFF: Same objection.

A. So there are -- there is definite evidence of damage to DNA, chromosomal aberrations, micronuclei that indicate damage to the nucleic acids. And that's in the tables. Those are in the tables.

And that's -- that's as far as -- we -- we -- if it was there, if there was linkages further down the line, we would have tried to look for that. Obviously, those 10 key characteristics are all points along that progression from the initial insult to actual tumor. These 10 key characteristics involved those steps. So we are looking for those steps. We are trying to make the linkage.

BY MR. GRIFFIS:

Q. Okay. And you found two?

A. We found two key characteristics of -- and those are genotoxicity and oxidative stress.

Q. Do you know of studies have been done looking at whether the actual presence of some of 10 key characteristics matches up with actual

carcinogenicity in multiple substances?

MS. WAGSTAFF: Objection to scope.

A. So there's -- what I understand is in group -- there are some group chemicals that exhibit at least two of the 10 key characteristics.

BY MR. GRIFFIS:

Q. And do you know whether large statistical analyses have been done matching up positive findings and the 10 key characteristics with whether a substance is a known carcinogen and finding that there is or is not a relationship between those two things?

MS. WAGSTAFF: Object to the form.

A. I haven't done that analyses.

BY MR. GRIFFIS:

Q. Okay. Do you know of anyone --

A. Analysis. I don't -- I can't recall. I don't know that. I know it's -- yeah. There's some data out there, but I'm not aware of it, exactly what it is -- where it is.

Q. Okay. As to the other eight characteristics -- and I'll run through them quickly just so you can remember what they are. And here's my question. As to other eight, IARC

working group 112, subgroup 4, either found that it doesn't appear to be applicable at all or found that the evidence was weak, which is the lowest classification you could give it, correct?

And that's -- shall I run through them?

A. The ten key characteristics -- or the other eight? Sure.

Q. Other than genotox and oxidative stress, found --

A. The others --

Q. -- no evidence or weak --

A. Or moderate. Maybe there was moderate. I don't remember. One of the key characteristics may have been labeled moderate, but I can't -- I don't recall exactly.

Q. We can -- I can point you to where it is -- each one is in the monograph if you would like. They're all no evidence or weak.

Act as an electrophile, altered DNA repair causing dynamic instability. That's two so far. Induce genetic alterations, chronic inflammation, immunosuppressive, modulate receptor mediated effects, immortalization, alter cell proliferation, cell death, nutrient supply.

A. Okay.

Q. So weak or no evidence as to those?

A. I will have to look at the monograph. I -- I don't remember --

Q. All right.

A. -- specifically those because our focus was on oxidative stress and genotoxicity.

(Exhibit No. 13-19 marked for identification.)

BY MR. GRIFFIS:

Q. Exhibit 19 is the monograph, sir. And if you'll turn to Page 77.

A. Okay.

Q. Left-hand column, the tiniest paragraph in the column. "Glyphosate is not electrophilic."

A. Yes.

Q. Okay. Next one, "Altered DNA repairs/cause genomic instability"?

A. Okay. Where is this?

Q. On 73.

A. Page 73.

MS. WAGSTAFF: Where on Page 73?

Q. 4.2.5, other mechanisms. We can take out several of them here. "No data on immortalization or genetic alteration, altered DNA

repair, or instability after exposure to glyphosate were available to the working group."
A. Okay.
MS. WAGSTAFF: Object to the form. It says were available.
BY MR. GRIFFIS:
Q. Working group found no evidence on those; is that right?
A. There -- well, no data available to examine those.
Q. Page 78. Weak evidence is at the top of the first column. "Weak evidence that glyphosate or glyphosate based formulations induced receptor mediated effects."
A. Okay. Yes.
Q. Weak evidence, next -- start of the next paragraph, "Weak evidence that glyphosate may effect cell proliferation or death." Next paragraph, "Weak evidence that glyphosate may affect the immune system, both the human and cellular response."
Next paragraph, "With regard to the other key characteristics of being a carcinogen, the working group considered that the data were too few for an evaluation to be made.

A. Yes.
Q. So do you agree with me that, other than genotoxic and oxidative stress, as to the 10 key mechanisms, the working group either found no evidence or found the evidence to be weak?
MS. WAGSTAFF: Objection. Misstates the record. I think you read that there was no data available in a few of those.
A. There was no data available to evaluate some of these key characteristics, or if there was, it was deemed to be weak evidence.
BY MR. GRIFFIS:
Q. Okay. You didn't have --
A. On the other key -- on those other eight. Either the data wasn't there or if there was data, it was deemed not to operate through that mechanism.
Q. And you did what you considered to be a comprehensive search to find any data that existed, right?
A. It was a -- yeah. Yes. Absolutely.
(Exhibit No. 13-20 marked for identification.)
BY MR. GRIFFIS:
Q. Okay. Exhibit 20.

MS. WAGSTAFF: Uh-huh (affirmative response).
BY MR. GRIFFIS:
Q. Sir, this is another document that you provided to us or that you provided to your lawyer and they provided to us perhaps. 112 mono 4 -- that's working group 112, monograph 4, mechanistic evidence summary.
And the first section is toxicokinetics; is that right?
A. Correct.
Q. Is the toxicokinetics section here something that you prepared?
A. I would have had prepared this, yes, as a summary of the -- of the section.
Q. Okay. So this is a document that you created summarizing the toxicokinetic information that you were finding?
A. Yes. This would have been the high points to highlight.
Q. All right. And you created this when?
A. This would have been created -- we created these summaries at the meeting.
Q. Okay. Key characteristics electrophilicity, glyphosate is not electrophilic.

We just found that in the monograph itself, right?
A. Correct.
Q. Okay. And genotoxicity -- and you wrote in, "In vivo evidence on genotoxicity of glyphosate largely" --
A. Can I clarify one point?
Q. Yes, sir.
A. I summarized the toxicokinetics. These key characteristics were -- I didn't -- I didn't make this part of the summary. I just -- whoever and I -- I just provided the toxicokinetic bullets.
Q. Okay. Who made the key characteristics section?
A. I don't recall. I don't recall. It may -- one of the -- one of the five of us who was on that subgroup.
Q. All right. It was sort of created at the -- at the working group 112 while you were in Lyon by someone in your group but not you?
A. Correct.
Q. Genotoxicity. It says, "In vivo evidence on genotoxicity of glyphosate is largely inconsistent in studies in rodents, and no

conclusions can be drawn from human studies due to mixed exposures to pesticides and other chemicals," correct?

A. That's what it says.

Q. Okay. "In vitro data in human and animal cells contain some evidence of genotoxicity of glyphosate and AMPA; however, a number of studies failed to observe evidence of genotoxicity."

I read that right?

A. Yes.

Q. "Positive studies for glyphosate, AMPA, and commercial formulations for glyphosate are available in a variety of plants, fish, and other marine organisms."

I read that right, correct?

A. Uh-huh (affirmative response). Yes.

Q. And then, "The majority of standard AIMS test bacterial strains were not affected by glyphosate or AMPA even in presence of metabolic activation," right?

A. Correct.

Q. Would you explain to the jury how an AIMS test works and what the role of metabolic activation is in an AIMS test?

A. So an AIMS test is a mutagenicity assay in which bacteria -- salmonella bacteria are exposed to the chemical of interest and whether there are DNA damage -- DNA damage that results in mutations resulting. The addition of the metabolic activation system is often used to bioactivate the chemical in question to a DNA reactive molecule.

Q. So this is a test that looks a step or two down the chain that we've been talking about from DNA damage on one end to actual mutations, and it finds whether there are mutations, both in the presence of the chemical being metabolized and not metabolized, right?

A. Yes. It's a mutagenicity assay using a prokaryotic organism, not a mammalian cell. A bacterial cell.

Q. And it's universally used by regulatory agencies as a critical cancer screening tool; is that right?

A. It is widely used.

Q. Okay. Do you know of anyone who doesn't use it?

MS. WAGSTAFF: Objection.

A. I don't know.

BY MR. GRIFFIS:

Q. Okay. All right. Now, during your discussions with group 4 -- subgroup 4, tell me what you discussed about the in vivo evidence on genotoxicity of glyphosate being inconsistent in studies in rodents.

What was inconsistent about the in vivo evidence on genotoxicity?

A. I don't -- this could -- this is an earlier draft. I don't recall what was considered inconsistent about it. There are tables with information on the in vivo evidence of genotoxicity in some rodent species. So I don't recall what was considered inconsistent about the studies.

Q. And do you consider that the group's opinion as to whether the studies were inconsistent changed over time?

A. There -- there was more evaluation occurring during the meeting.

Q. Did the --

A. There was more evaluation of the -- of the data.

Q. Did the group's opinion that the in vivo evidence on genotoxicity was largely inconsistent

in studies in rodents change?

A. It became stronger.

MS. WAGSTAFF: Object to summation.

BY MR. GRIFFIS:

Q. And what caused it to become stronger specifically?

A. So I don't know specific information about -- about this, but I know we were in the meeting. We're evaluating the data at the meeting. We're debating the data. It's not locked. It's not carved in stone when we get to Lyon. There's a debate that goes on, a peer review that goes on throughout the week. So things change. Things are in flux. This is -- there's scientific debate.

Q. Okay.

A. I -- so that -- it's whatever is in the final monograph is the final evaluation.

Q. And is it fair to say -- you know, and I understand that we're here to question you as a fact witness and what you remember, not necessarily what the other members of the group remember, sir.

But is it fair to say that what you remember is that the group's conclusion at some

point was that in vivo evidence on genotoxicity of glyphosate was largely inconsistent in studies in rodents. Over time, the opinion strengthened in favor of more consistency, and you don't remember specifically why?

MS. WAGSTAFF: I'm going to throw an objection in there as to foundation. That was the group's opinion. Dr. Ross testified he didn't write this and is not sure who wrote this. This could be the opinion of one scientist and not the entire subgroup.

A. So what you've got here, what you were able to get was before the peer review of the group. So we were charged with writing summaries, and further analyses would have taken place, debate. I do -- I do think I can say that the strength of the evidence of genotoxicity in nonhuman mammalian systems strengthened over the week.

BY MR. GRIFFIS:

Q. Well, the person who was in charge of drafting the genotox section was Frank LeCurieux as we've established, right?

A. I'm -- yes. I'm pretty certain about that.

Q. So was this Dr. LeCurieux's initial view, or was it the view of the group after some discussion at some point during the process?

A. I don't know who wrote this key characteristics section at this -- you know, I don't know who wrote it. Whether it was Dr. LeCurieux, I'm not sure.

Q. There was nobody who was tasked with writing all of these sections, correct?

A. The summaries?

Q. Yes, sir.

A. I was tasked with summarizing the toxicokinetics for each compound for each of these summaries.

Q. My point is that there was nobody who was tasked with writing a electrophilicity and genotoxicity and altered repair genomic instability and chronic inflammation or oxidative stress and receptor mediated and proliferation or death and immunosuppression and epigenetic effect and immortalization. This would have to be --

A. I don't know if it was done as a group or one individual person did each of these key characteristics. I -- again, because of my focus on toxicokinetics, I don't know the answer.

Q. In the initial drafting assignments, there was no one person who was in charge of all of that?

A. So --

Q. So this isn't somebody's first draft?

A. Well, this is someone's first draft of the summary.

Q. Of the summary after the group came together and talked, right?

MS. WAGSTAFF: Objection. Foundation.

A. This -- well, these were -- these were being drafted at the meeting.

BY MR. GRIFFIS:

Q. Could this be a summary of all of the first drafts?

A. It's possible. I don't really know. I don't know at what stage this was being -- at which stage this is at.

Q. Okay. What was said, to your recollection, about the position that no conclusions can be drawn from human studies due to mixed exposure pesticides and other chemicals with regard to genotoxicity?

MS. WAGSTAFF: Objection to you're asking questions, as Dr. Ross said he didn't

draft the key characteristics section of this document.

A. I can't speak to what was meant -- what was -- what this author was writing here because it became clear that there were some important studies in exposed humans that suggested or indicated a genotoxic effect.

BY MR. GRIFFIS:

Q. You're talking about the exposed people in Ecuador?

A. Columbia.

Q. Columbia. I got the border correct.

Those are the studies you mean, though?

A. That's in table 4.1.

Q. 4.1. Those are the studies you mean, not other ones?

A. I'm referring to Bolognesi.

Q. Okay. Now, but this was something that was discussed in the group? This genotoxicity stuff was discussed as the group's --

A. Yes.

Q. -- opinions evolved over time, right?

A. Yes.

Q. Okay. And so what I'm asking you is

what you recall the group discussing with regard to the position that no conclusions can be drawn from human studies due to mixed exposures to pesticides and other chemicals.

A. This is where --

MS. WAGSTAFF: Same objection.

A. -- I was so focused on the toxicokinetics that I don't know the specific details about that.

MR. GRIFFIS: Okay. Let's take five or ten minutes.

VIDEOGRAPHER: Off record at 3:00.

(A short recess was taken.)

VIDEOGRAPHER: Back on the record at 3:08.

BY MR. GRIFFIS:

Q. Okay. Sir, before the break, we were talking about Exhibit 20 which says in the section entitled genotoxicity no conclusions can be drawn from human studies due to mixed exposures to pesticides and other chemicals.

And you talked about how the evidence -- how the views of the group changed over time based on human exposures, and you specifically cited the Bolognesi study to me,

correct?

MS. WAGSTAFF: I'm going to object on using that key characteristic because he said he didn't know who wrote it, and he didn't even know it was a group opinion.

A. Well, I can say that the -- the -- an important study was the Bolognesi study because it dealt with exposure to glyphosate both before -- it indicated that there was evidence of genotoxicity being exposed to humans.

BY MR. GRIFFIS:

Q. In the monograph, sir, which I take it is 19, all right. Exhibit 19, monograph, Page 77. In looking at the right-hand column at the top, sir. The evidence for genotoxicity caused by glyphosate formulations is strong. And it says there was three studies of genotoxicity -- end points and community residents exposed to glyphosate based formulations, two of which reported positive associations, right?

A. Uh-huh (affirmative response).

Q. And those are the Bolognesi study -- the Bolognesi study and Tu Pas y Nino (phonetic) study; is that right?

A. Is that in table 4.1? Yeah.

Q. Yeah.

A. Pas y nino, yes.

Q. And it says that two of the three studies reported positive associations.

Do you recall discussing at subgroup 4 that the second pas y nino study -- 2011 study followed up on the first and found no lasting alterations?

A. It would have been discussed.

Q. Do you recall that discussion?

MS. WAGSTAFF: Objection. Foundation.

A. Sorry?

BY MR. GRIFFIS:

Q. Do you recall that discussion?

A. I don't.

Q. Okay. You don't recall that there was a first pas y nino study finding formation of some micronuclei that was associated with exposure to Roundup, and the second study looking for lasting damage found none?

MS. WAGSTAFF: Objection to foundation.

BY MR. GRIFFIS:

Q. Do you recall that?

A. I don't recall.

Q. Okay. We'll look at them then.

The one that you cited to me was the Bolognesi study, correct?

A. Yes.

Q. Okay.

(Exhibit No. 13-21 marked for identification.)

MS. WAGSTAFF: I would object to going through specifically articles in the fact that this was the subgroup's conclusion about glyphosate, and Dr. Ross is just one portion of that. He's sitting here in the context of a deposition. Asking him to go through scientific data I don't think was what was contemplated by the order.

BY MR. GRIFFIS:

Q. I'm sorry. Here you go, sir.

And when you cited to me before the break the Bolognesi study specifically as evidence of glyphosate causing genotoxicity damage in human beings, what was your -- what was the point of citing that work to me?

A. Because it showed in exposed humans -- humans that were exposed to glyphosate based formulations, that the level of genotoxicity immediately following the exposure was greater

than baseline levels that were taken prior to the spray of the glyphosate based formulation.

So there was evidence in an exposed population of genotoxicity caused by the -- by the agent.

Q. And what was the significance of that to subgroup 4?

A. So -- because it's evidence in vivo that glyphosate may cause damage -- genetic damage to cells within an exposed population.

Q. And what was the importance of the Bolognesi study to subgroup 4 in its conclusion that there was strong evidence of genotoxicity?

MS. WAGSTAFF: Object to form.

A. Because looking at exposed populations to an agent and seeing evidence of DNA damage is strong evidence that it is occurring, that it can occur.

BY MR. GRIFFIS:

Q. So the Bolognesi was one of the strong pieces of evidence that you were relying on for your conclusions?

A. Not the only piece.

Q. Yes, sir. One of the strong pieces?

A. One of the -- one of -- one of the

strong pieces of evidence.

Q. Was it the strongest?

A. I can't -- I'm not -- I can't say that. It -- there was a lot of weight on it because it's in an exposed population.

Q. Okay. Please --

A. In vivo -- in vivo, too.

Q. Please explain what -- okay. You said there's a lot of weight on it because, A, it's in an exposed population and, B, in vivo.

Would you explain to the jury the significance of those two points, please?

A. Because the mechanism may operate in humans. The mechanism of genotoxicity may be occurring in exposed populations.

Q. Okay. And why is that important to a finding of genotoxicity?

A. Because it's becomes the real world. It's a human population exposed to the agent, and these people had evidence of genotoxicity. So they're -- it's a real world situation.

Q. Did you read the Bolognesi study while you were at working group 112?

A. I have looked at it, yes.

Q. Okay. And did you do it before subgroup

4 came to its conclusions?

A. No, I did not.

Q. Okay. This was after you left Lyon?

A. Yes.

Q. Let's take a look at it.

All right. First of all, though, sir, do you know who in subgroup 4 did read and analyze this, other than obviously Dr. LeCurieux who drafted the genotoxicity section?

A. I believe that our subgroup chair read it.

Q. You believe Dr. Rusyn did, too?

A. Yes.

Q. Anyone else?

A. Not that I'm ware of.

MS. WAGSTAFF: Object to speculation. And I also object to questioning on this article. And I request that, if you're going to be asking him questions on this, that Dr. Ross take the time and read this article completely and refresh himself with it before questions are asked.

BY MR. GRIFFIS:

Q. I'm going to direct you to some --

MS. WAGSTAFF: And if you need to read

the --

BY MR. GRIFFIS:

Q. Yes, sir. I was about to say that. If you need to read any other part of article other than where I direct you to answer a question, please feel free to do so. I'm going to start on Page 994, sir.

MS. WAGSTAFF: Dr. Ross, do you need to read the entire article?

THE WITNESS: I'm familiar with it. I -- if he -- if there's a specific question that I'll need time to analyze, then I'll let you know.

BY MR. GRIFFIS:

Q. Okay. This is part of the discussion section. The discussion section starts on 992, but I'm over on 994. The right-hand column, the third paragraph.

And it's talking about something called BNMN. For the court reporter --

A. BNMN. It stands for binucleated cells with micronuclei.

Q. And that's what they are measuring in this study, right?

A. Yes. One of the end points.

Q. So the frequency of BNMN increased after spraying with glyphosate, but not consistently, correct?

A. Point to where you're -- which paragraph now?

Q. The first sentence of the third paragraph. Right-hand column.

A. Oh, right-hand column?

Q. Yes, sir. Sorry.

A. Okay. I see where you're at.

Q. The results of -- and it goes on to say, "The results obtained with a second sampling carried out immediately after the glyphosate spraying showed a statistically significant increase in frequency of BNMN in the three regions where glyphosate was sprayed. However, this was not consistent with the rates of application used in the regions," correct?

A. Yes. And this was pointed out in the monograph.

Q. And then the first sentence of the next paragraph says, "There was no significant association between self-reported direct contact with eradication sprays and frequency of BNMN," correct?

A. Yes. That's what it says.

Q. Okay. At the bottom of that same paragraph, "Decreases in frequency of BNMN and the recovery period after glyphosate spraying were not consistent."

And it gives an example, correct?

A. And these points were brought up in the monograph.

Q. The next sentence -- the first sentence of the next paragraph says, "Overall, these results suggest that genotoxic damage associated with glyphosate spraying as evidenced by the MN test is small and appears to be transient," correct?

A. This is a conclusion of these authors.

Q. And the authors concluded that -- the authors observed that the changes that they saw were transient, correct?

A. One of the communities still had -- one of the communities had lower levels four months after the spray compared to the four to five days' spray. So there was evidence of genotoxicity right after the spray, and four to five months later, that genotoxicity had -- was not apparent.

Q. Now, when genotoxicity is repaired by

the body, it's not leading to cancer, right?

A. What this paper suggested was there is evidence that genotoxicity, in three or four communities that were exposed to the glyphosate based formulation -- that there was a statistical increase in micronuclei immediately after the spray.

And what was strong about the study, in our opinion, was there were baseline samples taken immediately before the spray, and those same individuals were assayed four days after the spray, and there was a statistical increase in the micronuclei.

That was an important basis for putting a strength -- a strength descriptor on that -- on this particular study.

Q. In doing so, you were disagreeing with the conclusions of the authors themselves, correct?

MS. WAGSTAFF: Object to the form. Argumentative.

A. We were -- in this -- you know, the analysis that was being done by the major participants who had reviewed this data was that there was a statistical increase in the level of

DNA damage.

BY MR. GRIFFIS:

Q. The authors --

A. This was considered to be strength -- a strength to the study.

Q. What the authors said -- the authors of the study said -- I'm on Page 995, the second column, and the second sentence of the first full paragraph.

"Based on the applicable Bradford Hill guidelines, it is not possible to assign causality to the increases in frequency of BNMN observed in our study," correct?

MS. WAGSTAFF: Can you tell me where you are?

MR. GRIFFIS: Page 995, right-hand column, first full paragraph, second sentence.

MS. WAGSTAFF: Okay. Got it.

BY MR. GRIFFIS:

Q. That's what they said, right?

A. Yes. That's what's here.

Q. "There's a smaller frequency of BNMN and MOMN in the region of no pesticide use compared with the regions where pesticides, including

glyphosate, were used, which is consistent with other reports in the literature. Although, temporality was satisfied in the increase in frequency of BNMN after spraying, this response did not show strength as it was not consistently correlated with the rate of application.

"Recovery was also inconsistent with decreases in frequency of BNMN in the areas or eradication spray, but not in the area where lower rates were applied on sugar cane," correct?

MS. WAGSTAFF: Are you asking if that's what it says?

BY MR. GRIFFIS:

Q. Yeah. That's what it says?

A. Yes.

Q. Correct?

And then second sentence in the last paragraph of the article, "The smaller number of subjects recruited in this study and small amount of information about the exposure precluded any conclusions," right?

A. So, yes, that's what it says. However, the subgroup found that there was a statistically significant increase in micronuclei immediately following the spray application in these

individuals.

Statistically significant meaning there's a higher number -- statistically significant increase in the level of genetic damage immediately following the spray. This was -- this was considered important.

Q. And all other causes of this in people who were living near the Columbia/Ecuador border being sprayed from planes with glyphosate formulations, many of which being sprayed due to coca eradication -- were those all ruled by the study?

MS. WAGSTAFF: Objection. Argumentative.

A. I don't -- I don't know. Again, my area of expertise on this sub -- subgroup was to do toxicokinetics analysis. I am just telling you the subgroup was presented with this information that there was greater levels of genetic damage; that it was due to the glyphosate formulation being sprayed; and it was increased immediately following the spray compared to baseline values in the same individuals.

So there was evidence there that -- of genotoxicity that -- that was considered

strong.

BY MR. GRIFFIS:

Q. The two people in the group that actually read this -- that you know actually read this before the conclusions came out are Dr. Rusyn and the person who wrote the section, Frank LeCurieux. Correct?

MS. WAGSTAFF: Objection. I don't think he knows what everyone in the subgroup read.

A. Yeah. I don't know -- I don't know what else -- you know, I don't know about the other authors or the other participants. Whether they read it or not, I don't know.

BY MR. GRIFFIS:

Q. Okay. But --

A. But I know -- I do know that Mr. LeCurieux and Ivan would have read this.

Q. And did they say -- did you disclose in the IARC monograph that the authors of the paper didn't find there was any association?

MS. WAGSTAFF: Objection. The monograph speaks for itself.

A. Monographs -- it -- there's limitations that were described in the monograph.

BY MR. GRIFFIS:

Q. Did the disagreement with the conclusions of the authors of the article -- was that disclosed in the monograph?

MS. WAGSTAFF: Objection. The monograph speaks for itself. Argumentative.

A. I don't know. I don't -- I don't know if it is or not.

BY MR. GRIFFIS:

Q. Okay. Do you know Dr. Solomon, one of the coauthors of the Bolognesi paper?

A. I don't know him.

Q. Okay. Do you know that he said in a letter to editor -- I'm sorry -- in an interview that IARC got his study completely wrong?

A. I don't know that.

Q. Okay. Did anyone tell you that he was quoted as saying, "They got this totally wrong. They said the study showed there was relationship. It's certainly a different conclusion than the one we came to"?

MS. WAGSTAFF: Objection. Dr. Ross just stated he didn't know.

A. About -- about his comments? I don't know about those comments.

BY MR. GRIFFIS:
Q. Have you followed the discussions in the scientific community about IARC's methodology and IARC's conclusions followed you leaving working group 112?
A. I am aware of press, yes, regarding --
Q. Not this specific one, but some other press?
A. I don't recall this -- seeing this.
Q. And what have you followed?
A. I have seen reports in the Morning Consult and New York Times.
Q. Anything else?
A. I have seen some stuff in Huffington Post and Genetic Literacy Project and Monsanto's website.
MS. WAGSTAFF: I'm going to object about questions regarding what he's seen in the press regarding the 112, when the entire alleged purpose of this deposition was the working group mechanism's decision-making process, and what has happened since then in the media is completely irrelevant. And I believe that Judge Charbrio would agree.

BY MR. GRIFFIS:
Q. Have you been following those things yourself, or are these things that people e-mail you and you read when they happen to do that or what?
MS. WAGSTAFF: Same objection.
A. I've been familiar with it.
BY MR. GRIFFIS:
Q. Okay. Have any of the people -- and I'm talking about scientists who are commenting.
Have any of scientists who have commented in a critical way about IARC made any points that you considered to be useful or valuable critiques of the review that you did?
MS. WAGSTAFF: Objection. Once again, completely irrelevant and outside the scope of what the deposition allowed and requested.
A. I believe what we did was appropriate on -- based on the guidelines we were given in the preamble and -- yes. So I think what we did was appropriate. I can't comment beyond that.
BY MR. GRIFFIS:
Q. Okay. So you feel that you appropriately followed the guidelines that you were given?

A. Yes.
Q. Have you seen any criticisms of the guidelines that you were given you considered to be valid or fair?
A. No. I haven't -- no. I haven't seen criticisms of the guidelines we were given in the preamble that I felt were -- well, let me rephrase that. I haven't really seen criticisms of the guidelines.
Q. Okay. Fair enough.
Now oxidative stress. You said that you did a peer review of that section. It took about a day and a half of total time, including sending in the comments; is that right?
A. Yes.
Q. Okay. Now, without the oxidative stress findings, what would the mechanism group's recommendation have been?
MS. WAGSTAFF: Objection. That calls for speculation, and it's a hypothetical when the subgroup actually did find oxidative stress in its totality of the evidence type recommendation. And I don't think that anything -- any response would be anything more than speculation.

A. I'm not sure I understand the question.
BY MR. GRIFFIS:
Q. Yes, sir. I'm trying to understand how critical the oxidative stress findings were as compared to the genotoxicity findings in your conclusions that there was strong evidence that mechanisms existed by which glyphosate could cause cancer supporting, at one point, an upgrade which you didn't end up needing to advocate, et cetera.
How critical were the oxidative stress findings as compared to the genotox findings?
MS. WAGSTAFF: Again, I'll object to the fact that you're asking him to speculate on a hypothetical that never happened.
A. In terms of the 10 key characteristics, they were equally important.
BY MR. GRIFFIS:
Q. There's no hierarchy in the 10 key characteristics?
A. I'm not familiar with one.
Q. Okay. Are they considered all to be equal markers of carcinogenicity?
A. I don't think I am the one who can answer that.

Q. Is anyone in the mechanism group one who can answer that?
A. I think they are all given equal weight, in general. There's a -- yeah. I can't say there's one given more weight than the other.
Q. Okay. When you said, "I'm not the one to answer that," did you have someone in mind who --
A. No.
Q. -- would be better able to answer that?
A. I think a cancer biologist might be more appropriate to answer that specific question. We -- I looked at these 10 key characteristics as all being equal. We are trying to find the body of evidence that falls into each one of these key characteristics. What is the totality of the peer reviewed, published, openly available literature. So I don't think there's any bias in terms of one over another.
Q. Okay, sir. Tell me if this is right, then, that a cancer biologist may be better able to comment on the relevance of any particular one of the 10 key characteristics to formation of cancer.
Your mission was different. It was

to put the evidence into the bins and assess whether there was medium, moderate, or strong evidence with regard to each of the bins, correct?
MS. WAGSTAFF: Objection to form.
A. My job was to evaluate the toxicokinetic data on glyphosate.
BY MR. GRIFFIS:
Q. And group 4's job --
A. Group 4's job was to work on toxicokinetics, which I was primarily responsible for, and to evaluate the data -- the database on these 10 key characteristics.
Q. So group 4's mission was to put the evidence into the bins, into the ten categories, and assess within each bin whether it was weak, moderate, or strong evidence or we have no data in some cases, correct?
MS. WAGSTAFF: Object to the form. Use of the word "mission."
BY MR. GRIFFIS:
Q. Is that correct, sir?
A. Yes. Their -- yes.
Q. Okay.
(Exhibit No. 13-21 and Exhibit No. 13-22 marked for identification.)

MS. WAGSTAFF: Did you mark the Bolognesi as 21, or do you want to?
MR. GRIFFIS: I think so, yeah.
MS. WAGSTAFF: Okay. This will be 22.
MR. GRIFFIS: Yes.
MS. WAGSTAFF: I'm going to object to using the exhibit considering we can't read 95 percent of it.
BY MR. GRIFFIS:
Q. Exhibit 22, sir, is an e-mail from Ivan Rusyn that you produced as part of your production to Lauren Zeise, Frank LeCurieux to you, and -- I can't read the last one.
MS. WAGSTAFF: Was it produced by --
BY MR. GRIFFIS:
Q. What I want to ask you about is the big thing, not the little one. I mean, the rest of this that's very hard to read is primarily a list of assignments -- or recapitulation of the assignment list.
What I want to ask about is this large legible chart that Dr. Rusyn sent to members of the subgroup 4.
MS. WAGSTAFF: Object to foundation of this document.

BY MR. GRIFFIS:
Q. With regard to mechanistic, do you see the three squares at the top -- three rectangles, cancer in humans, cancer in experimental animals, and mechanistic and other relevant data?
A. Yes.
Q. Okay. And with regard to mechanistic and other relevant data, which, of course, was the portion that your group was focused on, there are dotted lines blowing up some questions. "Identify, establish some likely mechanistic events." And then there's some questions relevant to that.
And, "Determine whether each mechanism could operate in humans," and there's a question for that.
Do you see that?
A. Uh-huh (affirmative response).
Q. Now, do you recall the purpose for which Dr. Rusyn sent this to you and the other members of group 4?
MS. WAGSTAFF: Object to using this document when you can't see the date. You can't see who sent it. You can't see who it was sent from.

And did Hollingsworth, LLP, blow this up, or was it produced --

MR. GRIFFIS: It was produced exactly like this. The smallness was exactly like this.

MS. WAGSTAFF: Okay.

MR. GRIFFIS: Dated February 10th, 2015. Sent to Zeise, LeCurieux, Ross, and my eyes fail me for the third.

MS. WAGSTAFF: I'll maintain my objection since we can't read this, but go ahead.

BY MR. GRIFFIS:

Q. Try to ask the question again?

A. Yeah. So...

Q. Yes, sir. There's three rectangles at the top -- cancer in humans, cancer in experimental animals, and mechanistic or other relevant data. You just said that that was -- of course, that was the area that group 4 was focused on.

And then there are these dotted lines that blow up some subpoints and questions relevant to mechanistic and other relevant data, right?

A. Correct.

Q. Okay. The question I asked was, do you recall the purpose for which Dr. Rusyn sent you and other members of the group this chart with questions?

A. This is before the meeting. We -- we were having a teleconference, I presume. And this was -- this is -- this looks like verbiage that comes from the preamble and how to address the mechanistic data.

Q. Okay. So you understood this to be some of the questions that you would be focused on originating in the preamble in doing your mechanistic analysis.

Is that fair?

A. That's what the preamble -- yes. It comes from the preamble.

Q. Okay. On the issue of -- I'm looking at the first -- first item. "Identify, establish likely mechanistic events" -- and the second question -- the second set of questions asked, "Has each mechanism been challenged experimentally? Does supression of key mechanistic processes lead to supression of tumor development," correct?

A. Yes.

Q. Okay. And do you know of any data looked at by working group -- working group 112 at all showing that supression of genotoxicity or supression of oxidative stress, the mechanistic processes that you identified, led to supression of tumor development?

A. By which -- by glyphosate or glyphosate formulations?

Q. Yes, sir.

A. So to my knowledge, there are no evidence that suppressing those two would lead to supression of tumor development. I am not aware of any studies that looked at that. We -- yeah. There are supression of oxidative stress by the use of antioxidants when we looked at glyphosate.

Q. But those just looked at oxidative stress end points and not tumor development, right?

A. That's right.

(Exhibit No. 13-23 marked for identification.)

BY MR. GRIFFIS:

Q. Okay. Exhibit 23, sir. This is an e-mail chain involving Frank LeCurieux, yourself,

Kate Guyton, Matt Martin, and Lauren Zeise and Ivan Rusyn, correct?

A. Yes.

Q. Okay. Later adding in Andy Shapiro. I would like to focus first on Kathryn Guyton's March 13th, 2015 e-mail. Header of which is at the bottom of the first page, and the text appears on the second page.

Okay. Tell me when you're ready, sir.

A. Trying to get a timeline of the day here. Okay.

Q. Okay. So, again, I'd like to start out with Kathryn Guyton's March 13th, 2015 e-mail. The header is at the bottom of the first page, and the text is on the second page.

A. Okay.

Q. And she calls subgroup 4 the dream team and says those are Kurt's words -- Kurt Straif, correct?

A. Kurt Straif, yes.

Q. Kurt Straif called subgroup 4 the dream team?

A. That's what's written in this e-mail.

Q. Is that the first time you saw that?

A. I've seen this e-mail before.
Q. That's not quite what I meant.
Is this the first time you heard group 4 be called the dream team when you saw this e-mail?
A. Yes.
Q. Okay. She thanks you for your contributions during the plenary session and then says, "We were all impressed that Matt Martin was able to quickly calculate P values for the CA trend cut to aid interpretation of bioassay data."
I read that correctly?
A. Yes.
Q. Okay. And CA means Cochran Armitage?
A. Yes. I believe so.
Q. Okay. What --
A. I'm not a biostatistician, but I believe that's right.
Q. All right. Now, what group was Matt Martin in?
A. He was in subgroup 4.
Q. And what was the bioassay data? What is that a reference to?
A. Could be one of the five compounds. I -- I can't say with certainty which one it was.

Q. Well, it's talking about an animal study, correct?
A. Well, it's talking about some animal --
Q. Animal carcinogenic study?
A. Yeah. Animal cancer bioassay. But the specific compound...
MS. WAGSTAFF: Object to foundation of this questioning. He's unsure if it's even relating to glyphosate.
A. I don't -- I don't know if it relates specifically to glyphosate or not in this context.
BY MR. GRIFFIS:
Q. Okay. First of all, let me ask you this. Were you aware of Dr. Martin performing calculations on animal group studies?
A. I was vaguely aware. There was some -- he does statistics. He was doing some work at the meeting. I don't know the specifics of the analyses or which compounds or which particular animal bioassays were being examined.
I don't know the specifics because my focus was so much on the toxicokinetics during this stage of the meeting, that I don't know which -- which bioassay he is referring to.
Q. Were you aware that, during working

group 112, a Cochran analysis bioassay was recalculated with regard to glyphosate?
MS. WAGSTAFF: Objection. Foundation.
A. I -- I can't remember specifically if it was for glyphosate. There were several compounds. It's possible. It's possible.
BY MR. GRIFFIS:
Q. This is a slightly different question than do you remember what Dr. Martin did. This is specifically asking about glyphosate.
Do you recall that a Cochran analysis bioassay calculation was performed with regard to glyphosate during working group 112?
MS. WAGSTAFF: Objection. Foundation.
A. I can't -- with certainty, I can't remember which one was being analyzed.
BY MR. GRIFFIS:
Q. Do you recall that that Cochran analysis -- I'm sorry -- the Cochran Armitage analysis done on a glyphosate bioassay resulted in purported statistical significance where it had not existed before?
MS. WAGSTAFF: Objection. Foundation.
A. I don't know the specifics of that.

BY MR. GRIFFIS:
Q. Is that something you recall from the plenary sessions or from the other discussions that you participated in or heard?
A. I wasn't in subgroup 3, so I -- I don't know the specifics. I wasn't in their conversations about the statistical tests.
Q. Other than Matt Martin and Christopher Portier, who do you know who was performing statistical analyses during working group 112?
MS. WAGSTAFF: Objection.
A. I don't even know if Chris Portier was. I don't know.
BY MR. GRIFFIS:
Q. Do you not know that Chris Portier was?
A. I don't know.
Q. Okay. And you told us he was there as the bio statistician. Correct?
MS. WAGSTAFF: Object to the form.
A. Yes.
BY MR. GRIFFIS:
Q. Did he spend time with groups other than working group four? I'm sorry. Subgroup four?
A. I don't know if he spent time with them.
Q. Was he present at all subgroup four

meetings?
A. Oh. I think there was one point he had to step out. I don't remember which point.
Q. Okay.
A. There was a -- I can't -- he wasn't 100 percent there.
Q. Okay. One session he stepped out?
A. Yes.
Q. Okay. Other than that --
A. I recall that.
Q. Other than that, he was in all of your meetings?
A. Other than that, yes.
Q. Okay. This document mentions IARC table builder. Okay. Correct?
A. This e-mail?
Q. Yes.
A. Uh-huh (affirmative response).
Q. Okay. And do you know what the IARC table builder is?
A. Yes. I didn't use it, but it -- it was there to present data in the tables that you see in the monograph.
Q. Okay.
A. But I didn't use it.

Q. Was it connected to IOPS or HAWC or any other particular system?
A. I believe it is in IOPS. Maybe in HAWC. I don't think so. It was -- I think it was IOPSs.
Q. So in the IARC, the way it works, you enter bioassay incidents data and it automatically runs peer wise end trend analyses and presents that data?
A. I don't know anything about that.
Q. Okay.
A. I don't know how it -- how that works.
Q. Do you know or would we have to ask someone else, whether both peer wise and trend, trend Cochran Armitage test are appropriate for all bioassay incident data?
A. It is not my expertise area. I believe both were used.
Q. Do you know whether they are used under different circumstances, different sorts of data, different rarities of end point et cetera or do you not know?
A. I don't -- I don't know the details of that. I'm not with the peer wising and trend, I don't know when is the most appropriate to use. I know in cancer bioassay data it is often used.

Both types of tests.
Q. Okay. You don't know when to pick one and when to pick the other --
A. That would be out of my area.
Q. That's fine. And to the first e-mail in this document, the one from Katherine Guyton. Frank LeCurieux is cc'ing you March 13th of 2015. She is responding to a suggestion, Mr. LeCurieux, to involve subgroup one and more analyses. That's not the thing I want to focus on. She says a great suggestion.
And she says, "Unfortunately, I among other toxicologist don't understand the epidemiologist and their exposure compadres. However, I agree that their input, whatever it meant on the Bolognesi study, which was critical and in the end as valuable as, quote, sheep dip, with a monkey face?"
Would you explain what is meant by the input of the epidemiologist on the Bolognesi study?
MS. WAGSTAFF: Objection. This calls for speculation. Dr. Ross did not draft this e-mail. Dr. Guyton drafted this e-mail and asking him to opine on what she meant is pure

speculation.
BY MR. GRIFFIS:
Q. I'm not asking you to opine on what she meant, Doctor. I'm asking you what input the epidemiologist had on the Bolognesi study during the deliberation of the working group 112? Or is this something that happened that you don't know anything about?
MS. WAGSTAFF: Also, objection to the fact that there were multiple Bolognesi studies.
A. I don't recall what -- what is being discussed regarding the epidemiologists. I could only speculate.
BY MR. GRIFFIS:
Q. Whatever --
A. What they were talking about. Confounders and so forth. So I -- it is not -- I don't recall specifically this.
Q. There are two Bolognesi studies. One is the one we've discussed previously in this deposition about people being sprayed at the Columbia Ecuador border, and the other is an animal study. Right?
A. I don't know about the other. The only

one I'm -- I'm really familiar with is that in -- the one we looked at earlier.

Q. Do you know about epidemiologist or exposure people being involved in giving critical input with regard to either of the Bolognesi studies?

A. They may have. I don't know the answer. How much input, I don't know.

Q. Okay. You don't know anything about that event or where it took place?

A. I don't remember any conversation about that. I can't recall it.

Q. Okay. Take a break.

VIDEOGRAPHER: Off the record at 3:56.

(A short recess was taken.)

VIDEOGRAPHER: Back on the record, 4:05.

BY MR. GRIFFIS:

Q. Okay. We made a little bit of a nest of documents I handed you. I'd like to talk to you briefly about Exhibit 3, which is the subpoena that we sent early in this process, asking you to produce some documents.

A. This is the one in September?

Q. Yeah. Sometime in that -- not in connection with this deposition. The one which

you responded ultimately by sending us some documents. Would you tell us what you did. Don't tell me what your lawyers did, but tell us what you did to respond to that.

A. So I did searches of my work computer. Key word searches, I think, were IARC, glyphosate Monsanto.

I don't know the specifics. It was in the subpoena itself. But whatever was in the subpoena, I would do key word searches to make sure I could pull up all of the word docs, which several early drafts that we had -- I had -- I had drafted. That was the word docs on my work computer. I -- as you know, I had a spiral notebook that I kept notes with, and I looked for the notes from the meeting. And I made photocopies of it. Scanned it to the lawyers. Provided all of the word docs and provided it to the lawyers. And, yeah, I think so -- that's what I did. I scrubbed my computer for the -- you know, for what I needed to provide.

Q. Okay. I'm going to ask a series of questions to, you know, explore that a little bit and see if I can exhaust the process.

Do you work -- did you work on --

do you have multiple computers? Have a computer at home? A laptop --

A. Yeah.

Q. -- use?

A. I have my own laptop. And I also provided any -- a lot of it was redundant. I -- but if there was any documents on my laptop, I also provided that as well.

Q. Okay. Let's first get the complete list of computers that you used.

A. So it was my work computer and a personal laptop.

Q. Do you have a computer at home?

A. No. No. Not my personal computer.

Q. Do you have a personal computer at home?

A. I'm sorry. My laptop --

Q. Okay.

A. -- might take -- that I use at home.

Q. Okay. The laptop serves as your home computer?

A. Yes. Yes.

Q. And you don't use any other computer or tablets or ...

A. No.

Q. -- anything? Devices of any sort?

A. No.

Q. And you searched both your work computer and the laptop for the terms. Correct?

A. Right.

Q. Okay. In what program did you run those searches?

A. This is the search engine, this -- first of all, I knew where most of the documents were located, but to make sure I didn't have something in a folder I wasn't aware of, I used the search functionality on my laptop and on my work computer. Whatever that's -- that operating system is. I don't remember but -- what that is.

Q. It was the operating systems search --

A. Yeah.

Q. -- function, not Microsoft Word search function, is it?

A. Not Microsoft Word. The actual thing that will allow you to find any document that has, say, for example, IARC in the text.

Q. Right. Now, on the subject of PDFs, PDF don't always --

A. Yes.

Q. -- aren't always searchable.

A. I looked for PDFs as well.

Q. How did you look for PDFs that might not be searchable -- scan them or something?
A. I went through all and -- don't even know if we had any PDFs. I'm not sure. I can't remember for sure. But I looked for everything that was there in my PDF folder. I think there is ways in IARC I can -- you can use asterisks and dot PDF like asterisks IARC, asterisk dot PDF to do searches that would capture that.
Q. Yeah.
A. Capture those file.
Q. Some PDFs are intelligible enough to the computer that you can run word searches and some are not.
A. I --
Q. Okay. Did you -- what did you do about e-mail?
A. E-mail. So I looked but I think our IT guys were the ones capturing all of the e-mails that you have that -- that were -- that were responsive to the subpoena. So the IT guys were responsible for getting those.
Q. Other than any e-mail addresses that you might use exclusively for personal business, how many e-mail addresses do you have?

A. Oh. I have two e-mail addresses. One a personal and one a work.
Q. And do you send and receive work e-mails on the personal one for convenience ever?
A. No. The Yahoo one, I don't. I don't. I don't use it for work.
Q. And the work one, you ran some searches and found e-mails yourself. Did you provide those to your lawyers?
A. I'm trying to recall. I was told that IT will capture all of the e-mails. I don't recall actually handing over any e-mail hard copy of print outs.
Q. Okay.
A. Because I assumed IT would be more effective than I would be.
Q. And by IT, you mean IT here at MSU. Correct?
A. Yes.
Q. Okay. All right. Do you know what -- did you give them the list of search terms? Or was it handled by someone else?
A. I think this is a -- it's pretty common that they would have the search terms under the subpoena that they would be looking for. And they

would go through that, but I'm not the IT guy so...
Q. Don't know?
A. Yeah.
Q. Okay. You talked about your notebook. And what you did for that. You took it and you found -- I take it you found relevant date range.
A. Uh-huh (affirmative response).
Q. And copied the pages within that range and sent them off to your lawyers. Correct?
A. Right.
Q. Do you recall any pages from that date range that I haven't shown you today?
A. I don't recall. I don't -- I don't recall. I think I captured -- captured the date range of the meeting. Yeah. So I don't think there was any other -- you may have something I can't remember photocopying, but I don't remember it.
Q. I don't have anything in mine.
A. Okay. I thought you had another surprise.
Q. No, sir. No more surprises, if there were any.
And paper files, paper documents,

do you have any other than the notebook pertaining in any way to IARC, glyphosate or Monsanto?
A. No.
Q. Okay. And do you have any -- way that you operate -- primarily electronically, do y'all print things out?
A. Primarily.
Q. Or do you print them and then throw away?
A. Well, there would have been some early drafts that I would have tossed in the recycle. Might have had a hard copy of it and I was reviewing it myself. I didn't discover -- I didn't find any hard copies to hand over.
(Exhibit No. 13-24 marked for identification.)
BY MR. GRIFFIS:
Q. Almost done here, sir. Exhibit 24. Okay. Exhibit 25.
(Exhibit No. 13-25 marked for identification.)
MS. WAGSTAFF: Objection. Beyond the scope of this document. It really has no bearing on the subgroups conclusion about glyphosate.

BY MR. GRIFFIS:
Q. Sir, exhibit 24 is an e-mail from Katherine Guyton to you and to other persons talking about the subpoenas that were issued by Monsanto seeking documents, the documents we've just been talking about. Correct, sir?
A. Yes.
Q. Okay. And when you received this, it was sent on April 1st of 2016, you saw that Ms. Guyton was telling you the position of IARC all draft documents and materials prepared by the working group in advance or during the in-person monograph group meeting are to be considered draft and deliberative. And she went on to say that IARC does not encourage participants to retain working drafts of documents after the related monograph has been published. Correct?
A. Yes.
VIDEOGRAPHER: Off the record.
(A short recess was taken.)
VIDEOGRAPHER: Back on the record.
BY MR. GRIFFIS:
Q. Okay. Mr. White has said while we were off the record, that he believes that the e-mail was sent -- Exhibit 24 was sent in response to an

open record request and not specifically that document production request.
But, when you received this, did he do anything about it?
A. Which e-mail?
Q. Exhibit 24. Yeah.
A. Let's see. Well, Mississippi State lawyers were involved at this point. So I was talking with the Mississippi State lawyers about what -- what I needed to do.
Q. Okay. Don't tell me what you said to them or what they said to you.
But I assume you sent this on to them?
A. Yes. Yes, I did.
Q. Did you delete any drafts or any other documents?
A. No.
Q. Exhibit 25 is a letter dated April 7th, six days later from another IARC officer to working group members talking about request for disclosure of documents that some members of the working group to include yourself, sir, had received.
And at the end it says, "For all of

the above reasons IARC request you and your institute not to release any documents in your or your institute possession relating to your work in the capacity as a member of the working group."
Other than sending this on to your lawyers, did you do anything in response to this letter?
A. I provided this to the lawyers here at Mississippi State. That was -- that was my step.
Q. Now, at one point you were concerned about -- you were asked to participate in working group 117. Correct?
A. Correct.
Q. At one point you were concerned about doing so given the pendency of these document requests and your perception that handing over the documents would possibly put you at odds with IARC interests. Is that fair to say?
MS. WAGSTAFF: Objection to scope. This deposition is to explore the mechanism, group, subgroups, conclusion about glyphosate. And whether or not he had any reservation about participating in monograph 117, which was years after 112 opinion is completely irrelevant and outside of scope.

BY MR. GRIFFIS:
Q. Go ahead.
A. So my concern was that I would be in a conflict of interest between IARC and Mississippi State, and therefore I felt that I should resign from volume 117.
Q. And Kate Guyton at IARC reassured you and said we don't view there being any conflict? Correct?
A. I had discussions with lawyers here at Mississippi State. Kate had discussions with lawyers at IARC that there was no conflict of interest to serve on volume 117.
Q. And you -- sorry. Go ahead.
A. Go ahead.
Q. Didn't mean to cut you off, sir.
And you were asked to serve as the chair of mechanism 117. Is this right?
A. I served as the subgroup chair for mechanisms, yes.
Q. Okay.
A. For volume 117.
Q. Okay. Do you recall writing to Kate Guyton, "I expect Ivan, our fearless leader, to be there. Dr. Rusyn is a tough act to follow."

A. Those -- yes, that is my e-mail.
Q. And what did you mean by that?
A. I have a lot of respect for Dr. Rusyn as a scientist.
Q. What did you observe at working group 112. I assume that's what you were referring to when you said, "Tough act to follow." Correct?
A. Yes. I --
Q. What did you observe Dr. Rusyn doing at working group 112 that made you say that?
A. Extreme rigor. Very rigorous person -- scientist.
Q. What do you mean by rigor?
A. Evaluating the data objectively, demanding evidence.
Q. Sir, I'm finished with my questions for the time being. I'm going to reserve the rest of my time to follow up with -- there's going to be some questions from Ms. Wagstaff. I hope you understand that I had a job to do and Monsanto had a job to do in sending you those requests and conducting this deposition. I hope you haven't felt oppressed or harassed by me or my due process any more than is absolutely necessary.
A. Everyone's got a job to do. I

understand.
Q. Thank, you sir.
VIDEOGRAPHER: Break. Off the record.
(A short recess was taken.)
VIDEOGRAPHER: Back on record at 4:52.
EXAMINATION BY MS. WAGSTAFF:
Q. Good afternoon, Dr. Ross. My name is Aimee Wagstaff, and I am an attorney who is representing several plaintiffs who allege they have been injured after a result to exposure to glyphosate. Are you aware of that?
A. Yes.
Q. Okay. And so your deposition was first noticed by Monsanto in the multi-district litigation out of San Francisco and then we cross-noticed that deposition. Are you aware of that?
A. I knew it was in San Francisco, and I think it's been consolidated. What I understand the case has been consolidated. Is that --
Q. I mean, that's -- I'm just meaning are you aware that we cross-noticed your deposition?
A. Yes.
Q. Okay. And you and I have never met before today. Correct?

A. Correct.
Q. We've never spoken on the phone together before today. Correct?
A. Correct.
Q. We've never e-mailed before today. Correct?
A. Correct.
Q. And, in fact, the first time I met you was when you walked into this deposition room this morning. Correct?
A. Yes.
Q. Okay. And Mr. Griffis showed you an e-mail that my partner, my law partner Katherine Forgie sent you, I believe, a couple of years ago. Do you remember that this morning?
A. I don't remember what exhibit it was but, yes. I remember the e-mail.
Q. Okay. And just to be clear, you've never spoken with Ms. Forgie other than that unilateral attempt to contact you. Correct?
A. Yeah. I've never spoken -- spoken with Katherine Forgie.
Q. Okay. And we searched our law firm e-mails for a response from you and didn't find any. And that would be consistent with your

recollections to. Correct?
A. Yes.
Q. Okay. So and you haven't spoken with anyone from the Miller Law Firm out of Virginia. Correct?
A. No.
Q. Okay. And you haven't spoken anyone from Weitz Luxenberg out of New York City. Correct?
A. No.
Q. Okay. Excellent. So let's take a look at your CV really quick, which has been marked as Exhibit 4. And I'd just like to go over this real quickly, if I could.
It looks like it was updated in May of '17.
A. Yes.
Q. Okay. So this is -- this was provided by your attorney a couple of days ago, so it's the most updated CV that you have. Correct?
A. Right.
Q. Okay. And it looks like you've got a Ph.D. from UC Irvine?
A. Correct.
Q. Correct. And a bachelor of science and

chemistry from Cal Berkley?
A. Correct.
Q. Is that correct? And then it looks like you've got -- that was in 1998 and 1989 respectively. Correct?
A. Yes.
Q. And so if you backtrack your four years of college, my math may be off a little, but you started studying chemistry somewhere around 1985?
A. Yes.
Q. Okay. And to -- to today, which is in -- today is May 3rd, 2017, so you've been studying chemistry for about 32 years? Something like that?
A. Yes. Date me, yes.
Q. Not to date you. Okay. And it looks like you have -- starting with 1987, was your first sort of teaching assistant job at Cal Berkley as -- in the chemistry stock room teaching assistant. Is that correct?
A. Right. I worked as both. In the chemistry stock room and as a teaching assistant while an undergraduate.
Q. Okay. Great. So your first teaching job, if you will, in chemistry, was 30 years ago?

A. Yeah.
Q. Okay. And that works all the way up to today where you are, it looks like, currently an associate professor at Mississippi State University. Correct?
A. Yes.
Q. Okay. And you were working the department of basic sciences and you were awarded tenure, looks like, in July of 2010. Is that right?
A. Correct.
Q. Okay. If you go to the next page. It looks like you've received a lot of awards. You've listed one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen awards or honors that you've received in the field of advanced education and or chemistry. Is that correct?
A. Correct.
Q. Okay. The first one again being back in 1986 and the most recent one was an award that you received in China in 2015?
A. Correct.
Q. Okay. And all of this is true and accurate and up to date. Right?

A. Yes.
Q. Okay. And then if you scroll down and it says, "Research FTE 70 percent," what does that mean?
A. FTE is a way we break out our research teaching and service at the University. FTE stands for full time equivalent.
Q. Okay. And so can I -- can I take that to mean that 70 percent of your time your are researching?
A. That's right.
Q. Okay. And then you've talked about your -- you list peer review publications and you split that up into publications since joining Mississippi State University and prior to joining Mississippi State University. Right?
A. Correct.
Q. And it looks like you've written three peer review publications since you joined the University. Right? Look at the bottom where your left hand is.
A. More than three since I've joined the University.
Q. Okay.
A. I had several since I joined the

University. Several peer review public. It starts Page 7.
Q. Okay. So I was just confused because these three aren't numbered and then you start at 64, so I didn't know. So you --
A. Those are -- so first one in preparation. So this is something we are about to submit. And the other two are currently under review. So they haven't been formally accepted.
Q. Okay. So it's fair to say, though, that you've written in 64 peer review articles?
A. Yes.
Q. Since you joined the University. Is that correct?
A. Yes. 64 minus 12. Yes. So...
Q. A lot?
A. Right.
Q. Regardless. Okay. And what's the significance of having a publication peer reviewed?
A. Oh. Peer review is important in terms of having independent scientist evaluate the data that you are trying to publish and determining whether the conclusions you draw are based on the data that's provided within the publication.

Q. Okay. And to be published -- well strike that.

So is it fair to say peer review is sort of a safety net to ensure that the integrity of the -- and the high quality of the literature?

A. Yes. A peer review is very important because you have anonymous reviewers -- your peers in your field reviewing the evidence, reviewing the data and determining whether the conclusions are sound, whether the methodology is -- is sound. And it's an important -- peer review is a critical aspect of the scientific enterprise.

Q. Okay. And generally speaking, non-published science is not peer reviewed. Is that correct?

A. Non-published science -- it -- well, to be peer reviewed, and to be accepted into a journal, you need that safeguard to evaluate the evidence. Non-published data, we -- no one ever --

Q. It is unknown?

A. -- it is unknown. It hasn't been peer reviewed. It may be out there, but it's not been peer reviewed.

Q. Okay. And then it looks like, if you

move on to your CV, you get to Page 8, you've written some book chapters, you've written some chapters for some books. Then you participated in two IARC monographs. Is that correct?

A. Correct.

Q. And we have talked about IARC 112, which is the monograph where IARC considered the carcinogencity of glyphosate. Right?

A. Correct.

Q. And then one, looks like you also participated in IARC volume 117 after 112 that did not consider glyphosate. Correct?

A. Correct.

Q. Okay. And I also saw in one of your e-mails that you were invited to sit on the FIFRA scientific advisory panel board by the EPA. Is that correct?

A. Yes. I have served on a FIFRA panel 2005 -- 2006 perhaps. It was on pirethrodes. It wasn't glyphosate related.

Q. Okay. But that's an invitation from the EPA --

A. That was an invitation from the EPA.

Q. Okay. And then it looks like you have gone through -- you have one, two, three, four,

four pages of either current research projects or completed research projects in your CV. Is that correct?

A. Correct.

Q. And then presentations, and meeting abstracts, I counted up sixty-nine, if you totaled your presentations, your abstracts. Does that sound -- you don't have it numbered, but does that sound about right?

A. It sounds appropriate.

Q. Okay. And then you get to the Page 18 of your CV. My CV is only one page by the way. I think I need to beef that up.

But you get to Page 18 and your professional development. And you've got one, two, three, four, five, six, seven, eight courses that you've taken to stay abreast of the current field that you are working in. Correct?

A. Correct.

Q. Okay. Active outside collaborators. I'm guessing those are people that you collaborate with that are outside of Mississippi State University?

A. That's right.

Q. Okay.

A. That's what I mean by that.

Q. And you've got that you collaborate with St. Jude's Children Research in Memphis, Tennessee. Correct?

A. Right.

Q. You collaborate actively with the College of Veterinary Medicine at the University of Georgia. Is that right?

A. Right.

Q. Okay. And then you also collaborate with Jing Xu Academy of Agricultural Sciences in China. Is that correct?

A. Right.

Q. Okay. And then we talk about -- then you talk about your -- the rest of your time, which I guess isn't necessarily the rest, but 15 percent of your time is spent teaching. Is that right?

A. Right.

Q. Okay. And you've talked about all of the graduate courses that you have taught. You have taught a graduate course in the mechanisms of toxic action molecular toxicology. Is that correct?

A. Right.

Q. Okay. You've also taught in organ systems toxicology one and two. Is that correct?
A. Right.
Q. You've taught a course multiple times in the mechanisms of toxic action?
A. Yes.
Q. Correct. And you've taught a course called the current literature in toxicology. Is that right?
A. Right.
Q. Okay. You guest lectured in CVM graduate courses. What's CVM?
A. College of Veterinary Medicine.
Q. Okay. And you lectured -- you guest lectured on pharmicokinetic in a pharmacology course. Is that correct?
A. Right.
Q. And these were all -- these guest lectures were invitations from the regular professor. Right?
A. Right.
Q. Okay. And then if you turn to Page 20, and I won't go through the list, but it looks like you have student and post doctoral advisements on several students that -- through your time as a

professor. Is that right?
A. Right.
Q. I would say a dozen or so. Does that sound right?
A. In that ballpark, yes. Yeah. Uh-huh (affirmative response).
Q. And then we get to your service, which is a -- on Page 21, which is 15 percent of your time as well. And we look at the external review panels that you've been on and you've been on one, two, three, four, five, six, seven, eight, nine external review panels. Does that sound right?
A. Yes.
Q. Okay. And some of those, it says, "That you're an invited member by the NIH study session." What is NIH?
A. Well, National Institutes of Health.
Q. Okay. And you were an invited member to sit on their external review panel when they looked at the systemic injury by environmental exposures. Is that right?
A. Correct.
Q. Okay. You were also an invited member of the Agricultural Health Study National Advisory panel in Maryland. Is that right?

A. Correct.
Q. And we've talked about that this morning. Is that correct?
A. Yes.
Q. In fact, you only went to one meeting -- testified --
A. It was March 1st through 2nd of 2012.
Q. And then you have a list of the review editorial board that you sit on for journals.
And it looks like that there are -- I didn't count those up but it looks like there are a lot of those that you sit on. Is that right?
A. Yeah. These are primarily as peer reviewer for all of these journals.
Q. Okay.
A. I am on the editorial board of journal called Toxics.
Q. Okay. So in parenthesis, does that mean how many times you've peer reviewed?
A. Yeah. That's -- yeah. That -- yeah. Roughly determines how many times I've reviewed for each of these journals.
Q. Okay. So I see numbers like one, four, two, sixteen, three, but if you add them all up, I

mean, it looks like you peer reviewed 30 or 40 times?
A. Oh, more than -- yeah, more than that.
Q. Fifty times maybe?
A. Yeah.
Q. You peer reviewed a lot of journals. Is that fair to say?
A. Yeah, that -- yeah. Yeah.
Q. Okay. And then you talk about your university service and your department and college service and your clinical diagnostic service and others. And then you give some references. Is that fair to say?
A. Yes.
Q. Okay. So after reviewing your CV, I think it's fair to say that you are very knowledgeable in molecular toxicology and probably considered an expert in your field?
MR. GRIFFIS: Objection to form. Irrelevant.
BY MS. WAGSTAFF:
A. Yes, I've been invited by panels and to review papers and by NIH study sections.
Q. Okay. So we spent the first five and a half hours of the deposition this morning going

through piece by piece and pulling out of IARC monograph 112 and pulling out certain pieces and analyzing them in isolation. Is that fair?

MR. GRIFFIS: Object to the form.

A. We have looked at various exhibits.

BY MS. WAGSTAFF:

Q. Okay.

A. -- related to volume 112.

Q. But the bottom line is that the IARC 112 determination was made by looking at the totality of the evidence. Is that fair?

A. Yes.

Q. Okay. And you would agree with me that there is not just one piece of evidence that drove that decision. Is that fair?

A. Correct.

Q. Okay. It was a totality of all of the evidence that was presented to the panel. Is that fair?

A. Correct.

Q. Okay. And you would agree with me, too, that the subgroup that you belonged to, which was the mechanism group for subgroup, also looked at the totality of the available evidence. Correct?

MR. GRIFFIS: Object to the form and

contrary to the testimony.

A. Looked at the totality of the peer reviewed publicly available evidence for mechanisms and toxicokinetics.

BY MS. WAGSTAFF:

Q. Sure. So if you look -- so you would agree me then that subgroup four, in determining that there was a strong association, looked at the totality of the toxickinetic evidence and also the totality of the evidence that was allowed to be looked at -- strike that. That was a horrible question.

So you would agree with me that work -- that subgroup four, in making its determination of a strong association, looked at the totality of the toxicologic evidence, as well as the published peer reviewed literature?

MR. GRIFFIS: Objection to form. Contrary to prior testimony.

A. It would -- I wouldn't strong association it. There was strong evidence for genotoxicity. There was strong evidence for oxidated stress. Two of the ten characteristics.

BY MS. WAGSTAFF:

Q. You're. And I stand corrected by saying

that.

So you would agree with me that when the subgroup four found strong evidence for genotoxicity and when subgroup four found strong evidence for oxidated stress, that subgroup four looked at the totality of the available evidence --

A. Yes.

Q. -- in making that determination?

MR. GRIFFIS: Object to the form. Contrary to in regarding available evidence.

A. Yes.

BY MS. WAGSTAFF:

Q. And you would agree with me that the available evidence includes the evidence as allowed by the preamble of the mon -- of IARC's monograph. Correct?

A. Yes.

Q. Okay. And you would also agree with me that there wasn't one particular piece of evidence that drove either of those determinations. Correct?

A. For oxidative stress and genotoxicity, no. It's not one study that drives it.

Q. Okay.

A. It's the totality of -- the overall coherence of the data basis.

Q. Okay. Excellent. And in looking at the totality of the evidence, working group -- IARC working group 112 found that glyphosate was a category 2 A probable carcinogen. Correct?

A. Yes.

Q. Okay. And that was unanimous vote by all working members. Correct?

A. Yes, it was unanimous.

Q. Okay. And similarly, the subgroup fours vote to make a strong -- showing of strong evidence for genotoxicity and for oxidative stress was also unanimous. Correct?

A. Yes. With an IARC, yes, it was.

Q. Within your group?

A. Within our subgroup.

Q. And can you explain for the jury, sort of in laymen's term, what oxidative stress means?

A. Yes. So oxidative stress refers to molecules that have unpaired electrons that are highly reactive and that can damage cellular macromolecule, such as lipids, proteins and nucleic acids.

They are produced during normal

cellular respiration. We produce it under normal situations. And in a normal cell, it could be exacerbated by environmental chemicals.

Q. Okay.

A. That is made worse.

Q. Okay. Can you tell me how much money you made for participating in IARC 112 panel review?

A. Oh. We need we -- we were not paid for volume 112. We didn't get paid. We got per diem and we had travel.

Q. So you didn't make any money?

A. We don't make money.

Q. Okay. And have you made any money since on -- from your working on -- strike that.

Let's look at the preamble. I forget which exhibit it's marked. I think it might be 10. Going off memory though. Okay.

MR. WHITE: Yes.

BY MS. WAGSTAFF:

Q. We have spoken a lot today about classifications that certain subgroups have made whether it be limited or whether it be sufficient. And these are definitions that IARC has put into the preamble. And we never went over those

definitions, so I would like to just make sure that the jury understands what IARC means when something is labeled limited or sufficient.

So if you could turn please to page -- of the preamble, if you could, please, turn to Page 19. And this is a section called evaluation and rationale. Right?

A. Okay.

Q. Okay. So we're looking at A, which is the carcinogenicity in humans. Correct?

A. Yes.

Q. Okay. And when something -- and this is also referred to as the epidemiology group. Correct?

A. Correct.

Q. Okay. And when something is limited evidence, when the epidemiology group labels it limited evidence, do you -- are you following with me on this?

A. Uh-huh (affirmative response).

Q. The actual -- the subgroup actually finds a positive association between exposure to the agent of cancer for which a causal interpretation is considered by the working group to be credible. Did I read that correctly?

MR. GRIFFIS: Objection. Beyond scope of this deposition.

A. That is correct.

MS. WAGSTAFF: I cross-noticed this deposition, so I get to ask questions but --

MR. GRIFFIS: I'm not talking about my scope. I'm talking about the discovery scope.

BY MS. WAGSTAFF:

Q. Okay. So, in fact, when the epidemiology group identify -- or classifies something as limited evidence, they've actually found a positive association that they find credible. Is that fair?

MR. GRIFFIS: Objection. Beyond the scope of this deposition and beyond Dr. Ross's knowledge since only working in group four, he testified many times.

A. But this is what is in the IARC preamble.

BY MS. WAGSTAFF:

Q. So that's fair.

A. It's in the preamble.

Q. Okay. So then if you move on, and you if you look down to B, which is the

carcinogenicity in experimental animals. Right? So now we're in the animal subgroup. We're still on Page 20.

Oh, and just to be complete on -- let me go back up. To be complete on the limited evidence in the epidemiology group, the definition is written in the preamble is a positive association has been observed between exposure to the agent, which in this case is glyphosate, and cancer for which a causal interpretation is considered by the working group to be credible, but chance bias or confounding could not be ruled out with reasonable confidence.

Did I read that correctly?

MR. GRIFFIS: Objection. Beyond the designated scope set by Judge Charbrio, beyond this witness' knowledge given his prior testimony.

A. That's what written.

BY MS. WAGSTAFF:

Q. Did I read that -- okay?

A. That is correct. It is written in the preamble.

Q. Okay. Excellent. And so if you move down to B where you look at the carcinogenicity in

experimental animals, in fact, working group 112 labeled it sufficient evidence. Is that correct? That was the final determination by the animal group?
A. Sufficient evidence.
Q. Okay.
A. Yes.
Q. And so can you read into the jury what -- what that means?
MR. GRIFFIS: Objection. Beyond the scope of this deposition as found by Judge Charbrio, beyond this witness' knowledge given his prior testimony.
A. Well, you know for from.
BY MS. WAGSTAFF:
Q. Read it.
A. From the preamble, "The working group considers that a causal relationship has been established between the agent and an increased incidents of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in A, two or more of species of animals or, B, two or more independent studies in one species carried out at different times or in different laboratories or under different

protocols." Should I read more?
Q. Nope. That's good.
And then if you look at -- there is a lot of discussion this morning with Mr. Griffis between the animal group determining whether to call it limited evidence or sufficient evidence. Do you remember that?
A. Yes.
Q. Testimony. Okay. So see let's look and see what definition means of limited evidence by the animal group. Okay. If you could please read that into the record on Page 21.
MR. GRIFFIS: Same objection as previously regarding scope. And this witness' testimony, he wasn't involved in any of those working groups. Three -- subgroup 3, also, just reading, a document speaks for itself.
BY MS. WAGSTAFF:
Q. Go ahead.
A. So this is from the preamble. "The data suggests a carcinogenic effect" --
Q. Okay. Hang on real quick. So limited evidence of carcinogenicity by the animal group still means that the data suggests a carcinogenic

effect. Right?
MR. GRIFFIS: Objection --
BY MS. WAGSTAFF:
Q. Keep going.
A. "But are limited for making a definitive evaluation because, A, the evidence of carcinogenicity is restricted to a similar experiment; B, there are unresolved questions regarding the adequacy of the design conduct or interpretation of the studies; C, the agent increases the incidents only of benign neoplasms or lesions of uncertain neoplasm potential or, D, the evidence of carcinogencity is restricted to studies that demonstrate only promoting activity in a narrow range of issues or organs.
Q. Okay. Excellent. You can put the preamble away. I think am done with questions about that for right now.
And I'd like to introduce as an exhibit -- are we on 26?
(Exhibit No. 13-26 marked for identification.)
Q. 26. Okay. The list of participants that you have referenced numerous times this morning. So this was the list of participants.

Correct?
A. Yes.
Q. Okay. This was the entire list of participants from the working group. Is that right?
A. Yes.
Q. Okay. And there you are, about three quarters of way down, Matthew K. Ross, Mississippi State University, United States of America. Is that right?
A. Correct.
Q. Okay. And if you go all the way down, invited specialist, there's Dr. Christopher Portier that we talked about numerous times today. Right?
A. Yes.
Q. And then if you go all the way down to the very bottom of the page, is Dr. Portier's conflict -- potential conflict of interest disclosure that you had referenced earlier today. Right?
A. Yes.
Q. Okay. And if you turn the page -- actually before you turn the page, it looks like within this -- this group, there's also a member

from the United States EPA, Matthew T. Martin. Is that correct?
A. Yes. He's one of the members.
Q. Okay. So is he doctor? Is it Dr. Martin?
A. Yes.
Q. Okay. So Dr. Martin was participating in monograph 112 as a member of the EPA. Is that correct?
MR. GRIFFIS: Object to the form. False.
A. He was -- he was member of the subgroup four. He was -- he was -- he was an employee of U.S. EPA.
BY MS. WAGSTAFF:
Q. Let me strike that.
And so Matthew T. Martin, while he was participating in monograph 112, was an employee of the United States EPA. Is that correct?
MR. GRIFFIS: Object to the form.
A. Yes. He was an employee of U.S. EPA.
BY MS. WAGSTAFF:
Q. And here on this list of participants, Matthew T. Martin is listed as being associated in

some way with the United States EPA. Is that correct?
A. Yes.
Q. Okay. And, in fact, Matthew T. Martin was part of the mechanism subgroup four that you are part of. Correct?
A. Correct.
Q. And that Matthew T. Martin, the United States EPA employee, was part of the subgroup that found a strong association with genotoxic and oxidative stress. Is that correct?
MR. GRIFFIS: Objection to the form. The bold -- at the top says these people not serving in any way representative of their governmental organizational which they are affiliated.
BY MS. WAGSTAFF:
Q. Is that correct?
A. He was a member of subgroup four.
Q. And subgroup four was the subgroup that found that there is a strong evidence for genotoxicity and for oxidative stress of glyphosate. Is that correct?
A. Yes.
Q. Okay. And so if you turn the page --

excuse me -- to the next page, it looks like representatives of national and international health agencies are listed there as well. And then you have observers and it look -- if you look a few down, it looks like Thomas Sorahan was there for Monsanto Company. Is that correct?
A. Yes.
Q. Okay. So Monsanto had an observer there during the working group. Is that correct?
A. Yes.
Q. Okay. Do you know Mr. Sorahan?
A. I do not know him.
Q. Okay. It looks -- if you look down at number four, it looks like he had said that he is a member of the European glyphosate toxicology advisory panel and received reimbursement of travel cost from Monsanto to attend Eurotox 2012. Do you see that?
A. Yes.
Q. Okay. And he's listed as being associated with Monsanto company in this participant list. Is that correct?
A. As an observer.
Q. Okay. And did -- were you aware that he was reporting back to Monsanto throughout the

course of the monograph working group?
MR. GRIFFIS: Objection. Foundation.
A. I wasn't aware of his communications.
(Exhibit No. 13-27 marked for identification.)
BY MS. WAGSTAFF:
Q. Okay. So I'm going to hand you an e-mail which is marked confidential, but it has already been publicly disclosed, so you don't need to sign a protective order.
But if you look at the second page, do you know who Donna Farmer is? You go to the bottom of the cascade. Yeah. Okay.
A. Where is she from? She's a Monsanto employee. I don't know Donna Farmer.
Q. Well, you see that her e-mail is donnafarmerat@Monsanto.com?
A. Yes.
Q. That would suggest she is affiliated with and an employee of Monsanto?
MR. GRIFFIS: Objection. Foundation. Beyond the scope of this deposition as designated by Judge Charbrio.
BY MS. WAGSTAFF:
Q. I will represent to you that she is a

Monsanto employee. Do you have any reason to doubt that?
A. No.
Q. Okay. And so she is writing to Thomas Sorahan, the Monsanto observer, the working group 112. Correct?
A. Yes.
Q. And this is on March 14th, which was a couple of days after the -- if I recall correctly the working group concluded on the tenth and/or 11th of March of 2015?
A. Tuesday -- I don't have the time line in front of me. I think that's the 10th.
Q. Okay. And so she -- so -- so Dr. Farmer asked Thomas Sorahan, as well with Christian Strupp, Matt Jensen and Bill Heydens, about the IARC findings at a CLA meeting on Thursday. And if you look at -- this e-mail is from Thomas Sorahan, if you look at the front page, when he is writing back to her.
MR. GRIFFIS: Objection as to any questions about this document. The witness was not on the document in any way. He's never seen it before. There's no foundation for its relevance. And this is beyond the

scope that was set by Judge Charbrio.
BY MS. WAGSTAFF:
Q. Okay.
A. I need to read this.
Q. Sure.
A. I haven't had a chance to read this.
Q. No problem.
A. From Donna Farmer. Just let me...
Q. No problem. Okay.
A. Okay.
Q. Ready?
A. Yes.
Q. Okay. So it looks like Donna Farmer was writing to some folks wondering why the information was released about the 2 A classification of glyphosate. Right?
MR. GRIFFIS: Objection. This is utterly speculative. This is a document that this witness has nothing to do with. He had to read it the first time. So question -- these questions would be better directed to Donna Farmer -- would have been deposed. This is just an attempt to put into evidence things that have nothing to do with this witness. Beyond the scope set by the judge.

BY MS. WAGSTAFF:
Q. All right. And I don't necessarily care about your answer to that question, so I can strike it if you want.
MR. GRIFFIS: I'll have the same objection to every question that you have about this document which has nothing do with --
MS. WAGSTAFF: I will tie it in. Don't worry.
BY MS. WAGSTAFF:
Q. So we've talked about the methodology of -- we spent the day talking about the methodology of monograph 112, and Monsanto's attorneys have done everything they possibly can do to try to knock down the creditability of monograph 112, so I'm tying this in to show what one of Monsanto's own employees said about the methodology of 112. And if you will let me finish my questions, I will tie that in. So, if you --
MR. GRIFFIS: Objection. Argumentative. Misrepresents the prior testimony. Misrepresents the course of this deposition. Demonstrates the improper use of the document. Witness -- nothing to do with this

document.
BY MS. WAGSTAFF:
Q. Okay. So it looks like Tom Sorahan, who was there as an observer for Monsanto, writes to Dr. Farmer and says, in the second paragraph, quote, "I know of -- I do know of instances where observers at IARC felt they had been treated rudely or briskly at monograph meetings. That was not the case for me at volume 112. I found the chair, subchairs and invited experts to be friendly and prepared to respond all comments I made." Do you see that?
A. Yes.
MR. GRIFFIS: Objection. Irrelevant --
BY MS. WAGSTAFF:
Q. Was that your experience --
MR. GRIFFIS: -- witness.
BY MS. WAGSTAFF:
Q. Was that your experience at monograph 112?
MR. GRIFFIS: Objection. Totally irrelevant. He wasn't there as an observer.
A. So what the question is -- what's -- ask me the question again.
BY MS. WAGSTAFF:

Q. Sure. The question is, did you feel that the chair and the subchairs and the invited experts were prepared to respond to all comments by the observers?

MR. GRIFFIS: Objection. No foundation. Observers -- or know how the observers were treated.

MR. WHITE: I will advise, Dr. Ross, again, that you only have to answer to the extent that you have actual knowledge.

A. I thought they were cordial.

BY MS. WAGSTAFF:

Q. Okay. And then if you look at the next paragraph, it says, "In my opinion, the meeting followed the IARC guidelines." Would you agree with that?

MR. GRIFFIS: Objection. This document is irrelevant to any issue that is relevant to the scope set by the judge. He's never seen it before. And it's not -- proper witnesses have already been deposed.

A. Yes. I felt the guidelines were followed.

BY MS. WAGSTAFF:

Q. Excellent. And then I'd actually like

to pull out Exhibit 13 that Monsanto's attorney marked this morning, please. Okay.

All right. So this is an e-mail that Monsanto's marked as an exhibit to this deposition. So I'd like to actually walk through what -- the genesis of this e-mail. If you need to take a minute to look at it please, please do. Tell me when you are ready.

A. Okay.

Q. Okay. So please tell the ladies and gentlemen of the jury who Katherine Guyton is.

A. Dr. Guyton was the responsible officer employed by IARC for the meeting.

Q. Okay. And so it looks like on this cascade if you go to -- up in the very top left when it says 5039. Looks like the last couple of pages are just signature blocks. So this e-mail starts -- you know, e-mails are kind of funky because they go backwards.

But this e-mail cascade starts it looks like on February 3rd of 2015. Correct?

A. Yes.

Q. Okay. And it looks like Donna Farmer and here's actually you can see -- there's her signature line, so you can actually see now who

Donna Farmer is -- on the toxicology or the product protection and nutrition lead for the toxicology nutrition center at Monsanto. You see that?

A. Yes.

Q. Okay. And so it looks like Donna Farmer, on February 3rd of 2015, is sending a list of material to the -- what was Dr. Guyton's role again? The --

A. She was the responsible officer for volume 112.

Q. Okay. So it looks like Dr. Farmer, on February 3rd, is actually sending material to the responsible officer of monograph 112 to be considered for the meeting. Is that -- and it looks like she is -- she is actually also sending it to an e-mail entitled monograph 112 at IARC.fr. Do you see that?

A. Yes.

Q. Okay. This was about -- about a month before the IARC met, the IARC committee members met in Lyon, France. Is that right?

A. Yes.

Q. Okay. And later that day, Dr. Guyton responds and says thank you for the information.

We will provide the appropriate scientific articles to the working group. Do you see that?

A. Yes.

Q. Okay. And then if you move to the next portion of the cascade, it looks like a few days later, Dr. Farmer from Monsanto again follows up with the -- Dr. Guyton from IARC and requests that confirmation that she received her e-mail and then she says, if you look at the bottom of the first paragraph, "I have also had a Kingston Flash drive with the zip files sent to you via FedEx international priority, which would be there typically in two business days." You see that?

A. Yes.

Q. Okay. So it looks like Monsanto was following up again and now they have priority two-day airmailed information and articles to IARC 112. Is that right?

A. Yes.

Q. Okay. And so then if you -- then if you keep going, you look at February 26th, which is one day later, so three weeks later, Donna Farmer from Monsanto again is writing to Dr. Guyton and giving additional information for the monograph 112. Is this correct?

A. Yes.
Q. So it's fair to say that Monsanto provided information to monograph 112 to be considered. Is that right?
A. It appears that they were sending information to IARC.
Q. Okay. And so if you look now -- this is where I'm going to start to bounce around a little. If you could look at the actual monograph, which I believe was -- I'm not sure -- what exhibit number was that.
MR. WHITE: 19.
BY MS. WAGSTAFF:
Q. 19. Okay. And if you turn to Page 46.
(Exhibit No. 13-27 marked for identification.)
BY MS. WAGSTAFF:
Q. Okay. Are you on Page 46?
A. Yes.
Q. Okay. And this is actually -- I'm sorry. Turn to Page 45. This is where the IARC actually talks about the Bolognesi paper that you spent some time talking about with Monsanto's attorney. Do you remember that?
A. Yes.

Q. Okay. And now I just wanted to show you -- put into prospective where we were. You see Bolognesi, et al, 2009 in the right hand column of Page 45?
A. Yes.
Q. Okay. And that's a discussion in the IARC -- the final IARC manuscript about that paper that you had discussed. Correct?
A. Yes.
Q. So if you turn now to Page 46, I just wanted to -- just wanted to confirm that some of the language that Monsanto's attorney was reading to you about the Bolognesi paper did in fact make its way into the monograph 112 paper as it was considered within the final evaluation. And where I would point your direction -- point your attention to is where it says, "However, comma, the increased infrequency of micronucleus formation."
And that is the language that you were discussing with Monsanto's attorney earlier. Correct?
A. Yes.
Q. Okay. So that information was considered and actually made it into the published

final documents. Is that correct? That's what we're reading, the final document. Right?
A. Yes. This, yes.
Q. So that information was considered in totality of the evidence in making the determination. Correct?
A. The issue -- this was the -- the point that was raised earlier about micronucleus formation observed immediately after Spring was not consistent with the rate of application used in the regions. So this is the -- the issue that was brought up by the Monsanto attorney.
Q. Right. And so --
A. And I made the point that that information is in the monograph.
Q. Excellent. So my question to you is -- and so -- by -- this may seem sort of self-explanatory. But by virtue of it being in the monograph final published paper, that suggests that it was, in fact, considered in the totality of the evidence determination that both the subgroup four and monograph 112 made. Is that correct?
A. Yes.
Q. Okay. And then I'd like to -- okay.

Okay. I'd like to --
MS. WAGSTAFF: This is actually highlighted so I'm only going to give you guys one copy.
BY MS. WAGSTAFF:
Q. Okay. This is an article that is from Bolognesi in 2010. And if you turn to -- this was produced to us by Monsanto, which is why they are Bates labeled below. But if you turn to the end of the Bates labels being 294, last three -- 294. Okay.
And on the left hand column, the end of the first paragraph, it says, "Results showed significant increase in MN frequency after glyphosate exposure, mainly when it is applied for maturation of sugar cane."
A. I've just got to find where you are at here.
Q. You want to look at -- where I highlighted, it will help.
MR. GRIFFIS: Object. The question about this study which is not one that foundation -- been laid was considered by the witness or anyone else in connection with group four deliberations.

A. Let me just read through this.
MR. GRIFFIS: Calls for expert testimony.
A. Let me just read this paragraph here.
BY MS. WAGSTAFF:
Q. Sure.
A. Okay. I've read it.
Q. All right. So do you see where it says, "Results showed significant increases in MN frequency after glyphosate exposure, comma, mainly when it is applied for maturation of sugar cane." Do you see that?
MR. GRIFFIS: Same objection. It is beyond the scope set by Judge Charbrio. Asking this witness to make comments, extra testimony on study unrelated to the glyphosate 112 monograph.
A. I see -- I see that.
BY MS. WAGSTAFF:
Q. Okay. And this is the same Bolognesi who wrote the article in 2009. Correct?
MR. GRIFFIS: Same objection.
A. I believe so.
BY MS. WAGSTAFF:
Q. Okay. Put that aside.

Do you know a Dr. Jim Perry?
A. No.
Q. Okay. Do you know if during the IARC monograph 112 meeting that the panelists considered Dr. Perry's report that he commissioned for Monsanto?
MR. GRIFFIS: Objection. Irrelevant beyond the scope of this deposition.
A. I am unfamiliar with the name and any data he -- any report he was commissioned.
BY MS. WAGSTAFF:
Q. Okay. And so earlier today, Monsanto's attorneys tried to whittle down the amount of time that y'all spent on this monograph. And they were trying to suggest that you spent 20 percent of a week on the glyphosate monograph. Did you remember that testimony?
MR. GRIFFIS: Object. Unfair characterization -- Dr. Ross who said 20 percent.
A. I remember the testimony.
BY MS. WAGSTAFF:
Q. Okay. But this is all related to work that you do every day. Correct?
MR. GRIFFIS: Objection. Vague.

Q. I'll strike that.
A. Rephrase your question. In terms of juggling acts?
BY MS. WAGSTAFF:
Q. No. I will rephrase. Okay.
An hour that you spend --
A. Yes.
Q. -- with your expertise, education wise and experience is different than an hour that someone without that expertise spends on this type of work. Correct?
A. Yes. Yeah, it's fair to say.
Q. Okay. I don't have any advance degrees in chemistry, toxicology or any of the things on your CV. So I'm guessing that an hour that you spend on that is way more productive than an hour I spend on that. Is that correct?
MR. GRIFFIS: Objection. Vague.
A. I would, yes.
BY MS. WAGSTAFF:
Q. It's fair to say that.
Okay. I told you that we weren't going to have any more questions on the preamble, but I do have one more question. If you could please pull that up. Which I believe is Exhibit

10.
A. 10.
Q. 10.
A. Okay.
Q. Okay. Can you point to me the place in the preamble where it says that the procedure that the IARC members follow must be a procedure set forth in a peer reviewed public literature? And I'm not talking about the data that you -- that you need to analyze.
I want to know where in the preamble it says that the procedure followed must be that within a published literature. And I will submit to you that I don't think that it does say that.
MR. GRIFFIS: Objection. Relevance.
A. Looking for peer reviewed public literature?
BY MS. WAGSTAFF:
Q. No. I am -- so I know that the preamble says that the IARC panelists must consider -- the data it must consider must be published literature available in the public domain. I know that. I'm just wondering -- the procedure I'm actually talking about, the ten factors that we talked

about that the mechanism group looked at.
Monsanto's attorney seemed to make a distinction that the procedure wasn't in published literature until after the monograph happened. So I'm wondering, is there anything in the preamble that requires your procedure to be in published data?
A. Okay. Right. I got you, what you're saying now.
Yeah. So in the -- in the preamble, under the mechanistic and other relevant data, section four, there's nothing in the preamble that states that examining the 10 key characteristics that that evaluation was published. There is nothing in there about that.
Q. Okay. And there's nothing in there that says that for procedures go, in any procedures --
A. As a procedural matter.
Q. Yeah. Okay. In fact, genotoxic and oxidated stress were known causes of cancer in the peer review literature prior to IARC. Right?
MR. GRIFFIS: Objection.
Mischaracterized the testimony.
BY MS. WAGSTAFF:
Q. Okay. Let me ask you -- let me restate

that. Prior to -- that was a bad question. Okay.
Prior to monograph 112, okay, so we're going right before that. The peer review literature recognized genotoxicity and oxidative stress as causes of cancer. Correct?
A. There were studies that indicated genotoxicity and oxidated stress by glyphosate -- caused by glyphosate.
Q. Okay. Thanks. And as much as Monsanto tried this morning to make IARC 112 and subgroup 4 the Dr. Ross show, it wasn't. It was a team effort. Right?
MR. GRIFFIS: Objection to the characterization. Misstates the whole day.
A. Yeah.
BY MS. WAGSTAFF:
Q. Mean your --
A. Yeah. I had -- my main focus in this monograph was to evaluate the toxicokinetic data for glyphosate and the other four compounds. It was to evaluate the toxicokinetic data and report on that and be a member of the subgroup four mechanistic, mechanisms subgroup.
Q. Okay. Excellent. And your co-subgroup members are experts in their own right. Correct?

A. Yes.
Q. I mean to get up to become a member of an IARC panel, you must be an expert of some sort?
A. Yes.
MR. GRIFFIS: Objection. Beyond Dr. Ross's knowledge. Foundation.
BY MS. WAGSTAFF:
Q. And so -- and so it is absolutely appropriate, you would agree with me, that you rely on your comembers analyses of studies. Correct?
A. Yes. That's very important.
Q. Right. I mean they didn't -- no one called up Dr. Ross and said, Dr. Ross, make this opinion all by yourself. Correct?
A. Right.
Q. Okay. And so it's very appropriate, you would agree, that you didn't read every single article, and, in fact, relied on your co-panelist, who are who co-experts in their analyses? Correct?
A. Yes.
Q. There's nothing abnormal about that. Correct?
A. No.

Q. And that is, in fact, what you do in the scientific world in a setting like this. Correct?
A. Correct. Absolutely.
Q. Okay.
MS. WAGSTAFF: Let's take like a two or three minute break. I may be done. Real quick. I just want to talk with Jeff.
VIDEOGRAPHER: Off the record at 5:46.
(A short recess was taken.)
(Exhibit No. 13-28 and Exhibit No. 13-29 marked for identification.)
VIDEOGRAPHER: Back on record at 5:53.
BY MS. WAGSTAFF:
Q. All right. I'm going to try to wrap this up in just a few minutes.
Why did you participate? Why -- strike that. Why did you agree to participate in monograph 112?
A. I have a lot of background in research experience in pesticide metabolism, pharmicokinetic, organophosphorus, pesticides in particular. So I felt I was -- I was well qualified to serve on the panel.
Q. And did you consider the invitation a prestigious invitation?

A. Yes.
Q. Okay. And would you agree with me that scientific debate is a good thing?
A. Yes.
Q. Okay. I'm going to hand you as my hopefully last exhibit of the day, a document that Monsanto's attorney referenced this morning and it may actually be an exhibit. I'm not sure if you actually marked it as an exhibit.
I tucked under here -- can I have one of those copies back? Sorry.
This is an article that was published in a journal. Correct?
A. Yes.
Q. Okay. And it looks like it was -- there are 94 authors of this article. Right?
A. Yes.
Q. And you are number -- you are in there.
A. Yep.
Q. You're number --
A. 68.
Q. 68th, correct? You're the 68th author. And are you familiar with the contents of this article?
A. Yes.

Q. Okay. And as we sit here today, do you still stand by the contents of this article?
A. Yes.
MR. GRIFFIS: Objection. It is irrelevant to this deposition. And this article you objected to on the grounds that it postdated IARC beyond the scope of the judge's designation extent that is correct, your questions are out, too.
BY MS. WAGSTAFF:
Q. And is anything -- strike that.
In March of 2015, you believed based on the totality of the evidence that glyphosate was a probable carcinogen. Is that correct?
MR. GRIFFIS: Objection. Misrepresents the record.
MR. WHITE: You can answer within the scope of the IARC. You don't have to give a personal opinion.
A. The monograph, I think, speaks for itself. I was a member of the volume 112 team. And it was classified 2 A.
BY MS. WAGSTAFF:
Q. Okay. And is anything -- was anything

that was said today changed your mind on the decision that monograph 112 panelist came to?
A. No.
Q. Okay. Thank you. No further questions.
VIDEOGRAPHER: Off record.
(A short recess was taken.)
VIDEOGRAPHER: Back on record.
EXAMINATION BY MR. GRIFFIS:
Q. Sir, thank you for your time today. I have a few more questions on the subject of peer review.
There's a difference in the field of academic science, sort of science that you are normally involved in between peer reviewed and non-peer reviewed studies. Right?
A. There is a difference.
Q. The peer reviewed studies tend to be the better studies because they are good enough that they can be submitted to journals or good enough that when your peers look at them, they give sufficiently favorable reviews the journal would publish them. Correct?
A. The peer reviews system acts as a gatekeeper in a way. Quality control mechanism.
Q. And it's certainly not a single unitary

gate. Is that right? And what I mean by that, sir, is that there are journals of varying qualities and there are peer review processes of varying degrees of rigor?
A. I would -- yes, I would agree with that.
Q. There are some journals that are very prestigious, and you know that if something is published in one of those journals, it has been through a pretty good peer review process.
In contrast, there are some journals that aren't so prestigious and you may not have such confidence in the peer review process that things that are published and have gone to; is that fair?
MS. WAGSTAFF: Objection. Foundation.
A. So I don't completely agree with that.
BY MR. GRIFFIS:
Q. Tell me why.
A. Because you're assuming that what you think is a lower tiered journal with a low impact factor, every peer review of that article that comes through there is -- is flawed. And I don't think that's the case.
Q. I didn't mean to put those words into your head at all, sir. There are -- just that

there is certainly, in your mind, a hierarchy of journals and hierarchy of rigor of peer review. It may not be from good to bad, but from good to less good?

A. Yeah. We call those impact factors. The type of journal that we consider of high quality, high level versus lower impact factor journals.

Q. Now, the unpublished data, the stuff that is produced by academic scientists that doesn't get published, that hasn't necessarily been through any sort of review process or auditing process or procedure to make sure that it's good science. Is that fair?

MS. WAGSTAFF: Objection.

A. Unpublished -- unpublished data essentially doesn't exist in academic science. It doesn't exist. If it's not published, it doesn't exist. In the academic world --

BY MR. GRIFFIS:

Q. Academics. It may as well not exist, is that what you mean?

A. That's right.

Q. I mean, it does actually --

A. Sure.

Q. -- existence --

A. Doesn't exist because it's not in the peer reviewed published, published literature.

Q. It doesn't count for you. You don't consider it?

A. Yes.

Q. Okay.

A. It -- yes.

Q. You didn't mean that such things didn't happen? Certainly, there are studies that don't ever get published because they are not good enough. That's fair?

A. There are studies that don't get published because they are not good enough? Did they go through peer review or did they -- depends on did they go through peer review system.

Q. Right. So my --

A. And someone may have found a flaw in the analysis.

Q. I would like to talk about good laboratory practices, studies that are done under good laboratory practices, by contrast with unpublished academic things.

A. Uh-huh (affirmative response).

Q. That you said may as well not exist for

purposes of what academic scientist consider to be valuable information. GLP labs are certified by the government. Correct?

A. To my knowledge, they are.

Q. They go through a rigorous certification process. True?

MS. WAGSTAFF: Object to the form. Using the word "rigorous."

A. I believe so. You know. Working in a GPL, I know there are steps they have to take.

BY MR. GRIFFIS:

Q. There are multiple levels of audits, both audits by internal auditors and the auditors and the lab are also audited by external auditors. Correct?

A. Yes.

Q. Okay. Data collection analysis, statistical review of the data, all of that is prescribed and regimented and controlled by the GLP regulations. Correct?

A. Since I don't work in GLP, it was a long time ago, I can't really address the specifics of what is involved in the GLP studies.

Q. Okay. But you know that there are a large number of regulations about how the

laboratory conducts its practice about the collection of data and so on. You don't know exactly what those are?

MS. WAGSTAFF: Object to foundation.

A. Yes. I think so. I don't know all of the details about GLP. But -- but they are, I'm sure, because I worked in it, there are things that we have to do.

BY MR. GRIFFIS:

Q. Do you know, for example, that GLP regulations require that before a study can be conducted, the study plan, the methodology to be used, need to be written down?

A. Yes. I am aware of that.

Q. So, in academic medicine, you may or may not have a prior plan. It would be best practice to have a prior plan, but you may not. But in a GLP lab, you have to have a prior plan; that's the rule. Right?

A. Again, I'm not an expert in GLP.

Q. Okay. Do you know, sir, that GLP labs are -- there are guarantees built into the process, as a whole point of GLP, as to the methodology that's followed and that the methodology that was set out in advance was in

fact followed?
MS. WAGSTAFF: Object to the foundation of -- and the word of the use of word guarantees. There is no guarantee in that I don't think. So form and foundation.
BY MR. GRIFFIS:
Q. Go ahead, sir.
A. I don't know all of the details of the GLP requirements, and what's involved in that.
Q. Okay. Do you know -- are you familiar, sir, that in addition to GLP certification and the instance of GLP lab, companies like Monsanto are very heavily regulated with regard to the science that they generate?
MS. WAGSTAFF: Object to foundation.
A. I would presume if they are trying to get their products registered by EPA, they are -- they are regulated.
BY MR. GRIFFIS:
Q. Are you aware that EPA and other regulators in other countries set forth a list of the experiments that must be done to establish the safety and efficacy of products that are submitted for registration by companies like Monsanto?
MS. WAGSTAFF: Object to the foundation.

Form and scope of the question.
A. I don't know all of the regulatory tests that are prescribed, but I'm aware that there are some for sure. I don't know all of the details.
BY MR. GRIFFIS:
Q. You don't know which tests are prescribed, but you do know that some are?
A. Clearly. I worked in a contract lab that would have to submit data to a chemical company that would submit it to EPA. So I'm familiar with that.
Q. Okay. When we're talking about the regulatory battery of studies conducted by companies like Monsanto, and other registrants of glyphosate products, we're talking about highly regulated studies with methodologies set forth in advance with bioassays prescribed by the regulators conducted in GLP labs with multiple layers of auditing. Correct?
MS. WAGSTAFF: Object to the foundation. There's no evidence in front of the deponent that any of that is actually an accurate description of the regulation. Object to the form.
A. What is the best way to answer it?

MS. WAGSTAFF: Another objection is he's testified he's not a regulatory expert. So he's just speculating.
A. I know there are requirements that they have to meet for their products to be registered with EPA. I don't know the specific details of it.
BY MR. GRIFFIS:
Q. And the quality and rigor of GLP certified studies conducted for regulatory approval is a completely different universe than that of unpublished studies produced by academic labs. Fair?
A. Unpublished studies?
MS. WAGSTAFF: Object to foundation -- I mean foundation and object to the form. Completely different universe.
A. I don't know. I can't answer that question.
BY MR. GRIFFIS:
Q. There is a world of difference in quality between the two?
A. I would disagree.
Q. You believe the GLPs certified labs produce bad science?

A. No. I didn't say that.
Q. Okay. What do you mean?
A. You implied that unpublished data that an academic scientist might have was performed poorly.
Q. You told me earlier that -- what I was alluding to, sir, you told me a little bit earlier that unpublished data created by academic science doesn't exist, which you didn't quite mean literally. You meant it may as well not exist because it is not even considered. Correct?
A. That's correct.
Q. And by contrast, GLP registration data and both continues to exist and is considered by every regulator in the world in making very important assessments about risk and hazard. Correct?
MS. WAGSTAFF: Object to foundation. Every single regulator in the world relies on GLP and I object to that. Objection to form.
A. I'm not a GLP expert. I know there are very stringent regulations in GLP laboratories. That doesn't mean -- that doesn't necessarily mean that the experiments -- that the data is valid. I mean, it could be done poorly.

The experiments could still be done poorly in a GLP laboratory, the data quality could still be poor.
BY MR. GRIFFIS:
Q. There are controls to make sure that they aren't, though. Right?
MS. WAGSTAFF: Object to foundation. He said he is not a GLP expert.
A. Yeah. I'm not a GLP expert. Controls are important in science and when studies are peer reviewed, the peer reviewers are looking for whether appropriate controls were utilized in the experiments, whether appropriate quality control aspects were followed.
BY MR. GRIFFIS:
Q. And you don't know if the data is real?
MS. WAGSTAFF: Objection. Argumentative.
A. You don't know if the data is real?
BY MR. GRIFFIS:
Q. Yes, sir.
A. Oh, if -- when you're peer reviewing?
Q. Yes, sir.
A. Oh, you think it could be fabricated? Is that what you're indicating?

Q. It's conceivable on peer review because you aren't auditing the lab, not backing up the scientist in that way. Correct?
MS. WAGSTAFF: Objection. Hypothetical.
MR. WHITE: You don't have to answer any hypotheticals.
BY MR. GRIFFIS:
Q. There aren't controls in academic labs in a systematic way, the way they are in GLP labs to ensure data quality. That's fair to say, right?
MS. WAGSTAFF: Objection. Foundation.
A. Yeah. It's an interesting question because GLP requires a great deal of prescriptions you have to follow. And I'm aware of that.
BY MR. GRIFFIS:
Q. Okay. I will move on from that.
In the preamble, which is Exhibit 10 there. Can you pull it up, please?
A. Preamble?
Q. Yes, sir. Page 20.
MS. WAGSTAFF: Hold on a second.
BY MR. GRIFFIS:
Q. In the description of sufficient evidence of carcinogenicity, do you know why the

preamble calls for studies ideally to be conducted under good laboratory practices?
A. Let me see. I'm going to read, "An increase in the incidents of tumors in both sexes of a single species in a well conducted study ideally conducted under good laboratory practices can also provide sufficient evidence." Do I know why?
Q. Do you know why IARC states that it is willing in some circumstances to rely on a single well conducted study ideally conducted under good laboratory practices? Why it says ideally conducted in good laboratory practices?
A. I don't know if it says single study. Of a single species --
Q. In a well conducted study.
A. Yeah. Again, I'm not an expert in GLP that can answer that question. Why -- I don't think it gets more weight than an academic study -- a GLP study.
Q. IARC says ideally such a study would be conducted under good laboratory practices. Is that right?
A. That's what -- that's what a preamble says, yes.

Q. Thank you for your time today, sir.
MS. WAGSTAFF: No further questions for me.
VIDEOGRAPHER: Off record, 6:11.
(Ended at 6:11 p.m.)

CERTIFICATE OF COURT REPORTER

I, Todd J. Davis, Court Reporter and Notary Public in and for the County of Madison, State of Mississippi, hereby certify that the foregoing pages contain a true and correct transcript of the testimony of MATTHEW K. ROSS, as taken by me in the aforementioned matter at the time and place heretofore stated, as taken by stenotype and later reduced to typewritten form under my supervision to the best of my skill and ability by means of computer-aided transcription.

I further certify that under the authority vested in me by the State of Mississippi that the witness was placed under oath by me to truthfully answer all questions in this matter.

I further certify that I am not in the employ of or related to any counsel or party in this matter and have no interest, monetary or otherwise, in the final outcome of this matter.

Witness my signature and seal this the 5TH day of MAY, 2017.

_____

TODD J. DAVIS, CSR #1406

My Commission Expires:
March 27, 2021

ERRATA SHEET

Case Name:
Deposition Date:
Deponent:

Pg. No.	Now Reads	Should Read	Reason

_____

Signature of Deponent

SUBSCRIBED AND SWORN BEFORE ME
THIS ____ DAY OF _____, 2017.

_____

(Notary Public) MY COMMISSION EXPIRES: _____

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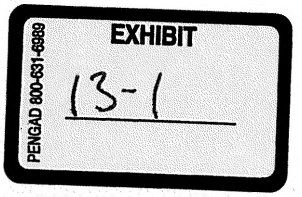
AO 88A (Rev. 02/14) Subpoena to Testify at a Deposition in a Civil Action

UNITED STATES DISTRICT COURT

for the

Northern District of California



IN RE: ROUNDUP PRODS. LIABILITY LITIG.

Plaintiff

v.

Defendant

Civil Action No. 16-md-2741-VC

SUBPOENA TO TESTIFY AT A DEPOSITION IN A CIVIL ACTION

To: Dr. Matthew K. Ross

(Name of person to whom this subpoena is directed)

☑ *Testimony:* **YOU ARE COMMANDED** to appear at the time, date, and place set forth below to testify at a deposition to be taken in this civil action. If you are an organization, you must designate one or more officers, directors, or managing agents, or designate other persons who consent to testify on your behalf about the following matters, or those set forth in an attachment:

Place:	Date and Time:
Mississippi State University 175 President's Circle, Allen Hall Mississippi State, MS 39762	05/03/2017 9:00 am

The deposition will be recorded by this method: video and stenographic

☑ *Production:* You, or your representatives, must also bring with you to the deposition the following documents, electronically stored information, or objects, and must permit inspection, copying, testing, or sampling of the material: See Exhibit A attached.

The following provisions of Fed. R. Civ. P. 45 are attached – Rule 45(c), relating to the place of compliance; Rule 45(d), relating to your protection as a person subject to a subpoena; and Rule 45(e) and (g), relating to your duty to respond to this subpoena and the potential consequences of not doing so.

Date: 04/21/2017

CLERK OF COURT

OR

Signature of Clerk or Deputy Clerk

Attorney's signature

The name, address, e-mail address, and telephone number of the attorney representing *(name of party)* Monsanto Company, who issues or requests this subpoena, are:

Eric G. Lasker, 1350 I Street NW, Washington, DC 20005; elasker@hollingsworthllp.com; (202) 898-5800

Notice to the person who issues or requests this subpoena

If this subpoena commands the production of documents, electronically stored information, or tangible things before trial, a notice and a copy of the subpoena must be served on each party in this case before it is served on the person to whom it is directed. Fed. R. Civ. P. 45(a)(4).

Civil Action No. 16-md-2741-VC

PROOF OF SERVICE

(This section should not be filed with the court unless required by Fed. R. Civ. P. 45.)

I received this subpoena for *(name of individual and title, if any)* _____

on *(date)* _____ .

❒ I served the subpoena by delivering a copy to the named individual as follows: _____

_____

_____ on *(date)* _____ ; or

❒ I returned the subpoena unexecuted because: _____

_____ .

Unless the subpoena was issued on behalf of the United States, or one of its officers or agents, I have also tendered to the witness the fees for one day's attendance, and the mileage allowed by law, in the amount of $ _____ .

My fees are $ _____ for travel and $ _____ for services, for a total of $ 0.00 .

I declare under penalty of perjury that this information is true.

Date: _____

_____
Server's signature

_____
Printed name and title

_____
Server's address

Additional information regarding attempted service, etc.:

Federal Rule of Civil Procedure 45 (c), (d), (e), and (g) (Effective 12/1/13)

(c) Place of Compliance.

(1) ***For a Trial, Hearing, or Deposition.*** A subpoena may command a person to attend a trial, hearing, or deposition only as follows:

(A) within 100 miles of where the person resides, is employed, or regularly transacts business in person; or

(B) within the state where the person resides, is employed, or regularly transacts business in person, if the person

(i) is a party or a party's officer; or

(ii) is commanded to attend a trial and would not incur substantial expense.

(2) ***For Other Discovery.*** A subpoena may command:

(A) production of documents, electronically stored information, or tangible things at a place within 100 miles of where the person resides, is employed, or regularly transacts business in person; and

(B) inspection of premises at the premises to be inspected.

(d) Protecting a Person Subject to a Subpoena; Enforcement.

(1) ***Avoiding Undue Burden or Expense; Sanctions.*** A party or attorney responsible for issuing and serving a subpoena must take reasonable steps to avoid imposing undue burden or expense on a person subject to the subpoena. The court for the district where compliance is required must enforce this duty and impose an appropriate sanction—which may include lost earnings and reasonable attorney's fees—on a party or attorney who fails to comply.

(2) ***Command to Produce Materials or Permit Inspection.***

(A) *Appearance Not Required.* A person commanded to produce documents, electronically stored information, or tangible things, or to permit the inspection of premises, need not appear in person at the place of production or inspection unless also commanded to appear for a deposition, hearing, or trial.

(B) *Objections.* A person commanded to produce documents or tangible things or to permit inspection may serve on the party or attorney designated in the subpoena a written objection to inspecting, copying, testing, or sampling any or all of the materials or to inspecting the premises—or to producing electronically stored information in the form or forms requested. The objection must be served before the earlier of the time specified for compliance or 14 days after the subpoena is served. If an objection is made, the following rules apply:

(i) At any time, on notice to the commanded person, the serving party may move the court for the district where compliance is required for an order compelling production or inspection.

(ii) These acts may be required only as directed in the order, and the order must protect a person who is neither a party nor a party's officer from significant expense resulting from compliance.

(3) ***Quashing or Modifying a Subpoena.***

(A) *When Required.* On timely motion, the court for the district where compliance is required must quash or modify a subpoena that:

(i) fails to allow a reasonable time to comply;

(ii) requires a person to comply beyond the geographical limits specified in Rule 45(c);

(iii) requires disclosure of privileged or other protected matter, if no exception or waiver applies; or

(iv) subjects a person to undue burden.

(B) *When Permitted.* To protect a person subject to or affected by a subpoena, the court for the district where compliance is required may, on motion, quash or modify the subpoena if it requires:

(i) disclosing a trade secret or other confidential research, development, or commercial information; or

(ii) disclosing an unretained expert's opinion or information that does not describe specific occurrences in dispute and results from the expert's study that was not requested by a party.

(C) *Specifying Conditions as an Alternative.* In the circumstances described in Rule 45(d)(3)(B), the court may, instead of quashing or modifying a subpoena, order appearance or production under specified conditions if the serving party:

(i) shows a substantial need for the testimony or material that cannot be otherwise met without undue hardship; and

(ii) ensures that the subpoenaed person will be reasonably compensated.

(e) Duties in Responding to a Subpoena.

(1) ***Producing Documents or Electronically Stored Information.*** These procedures apply to producing documents or electronically stored information:

(A) *Documents.* A person responding to a subpoena to produce documents must produce them as they are kept in the ordinary course of business or must organize and label them to correspond to the categories in the demand.

(B) *Form for Producing Electronically Stored Information Not Specified.* If a subpoena does not specify a form for producing electronically stored information, the person responding must produce it in a form or forms in which it is ordinarily maintained or in a reasonably usable form or forms.

(C) *Electronically Stored Information Produced in Only One Form.* The person responding need not produce the same electronically stored information in more than one form.

(D) *Inaccessible Electronically Stored Information.* The person responding need not provide discovery of electronically stored information from sources that the person identifies as not reasonably accessible because of undue burden or cost. On motion to compel discovery or for a protective order, the person responding must show that the information is not reasonably accessible because of undue burden or cost. If that showing is made, the court may nonetheless order discovery from such sources if the requesting party shows good cause, considering the limitations of Rule 26(b)(2)(C). The court may specify conditions for the discovery.

(2) ***Claiming Privilege or Protection.***

(A) *Information Withheld.* A person withholding subpoenaed information under a claim that it is privileged or subject to protection as trial-preparation material must:

(i) expressly make the claim; and

(ii) describe the nature of the withheld documents, communications, or tangible things in a manner that, without revealing information itself privileged or protected, will enable the parties to assess the claim.

(B) *Information Produced.* If information produced in response to a subpoena is subject to a claim of privilege or of protection as trial-preparation material, the person making the claim may notify any party that received the information of the claim and the basis for it. After being notified, a party must promptly return, sequester, or destroy the specified information and any copies it has; must not use or disclose the information until the claim is resolved; must take reasonable steps to retrieve the information if the party disclosed it before being notified; and may promptly present the information under seal to the court for the district where compliance is required for a determination of the claim. The person who produced the information must preserve the information until the claim is resolved.

(g) Contempt.

The court for the district where compliance is required—and also, after a motion is transferred, the issuing court—may hold in contempt a person who, having been served, fails without adequate excuse to obey the subpoena or an order related to it.

For access to subpoena materials, see Fed. R. Civ. P. 45(a) Committee Note (2013).

EXHIBIT A

DEFINITIONS AND INSTRUCTIONS

1. The term "Communication," as used in these Requests, is intended to have the broadest possible meaning and shall include any contact or act by which information or knowledge is transmitted or conveyed between two or more persons and includes, without limitation: (1) written contact, including but not limited to letters, memoranda, PowerPoint presentations, email, text message, telegram, telex, internet-based meetings, or other written or electronic documents or files; (2) oral contact, whether by face-to-face meetings, internet-based meetings, video conferences, telephonic conversations, or otherwise; and (3) nonverbal acts intended to communicate or convey any meaning, understanding or other message.

2. The term "documents" is used broadly, and encompasses all tangible things and recorded information possessed by you, whether such documents are located in computers, e-mail accounts, or hard-copy documents or files. The term "documents" includes, but is not limited to, handwritten, typed, or printed papers, whether in final or draft form, handwritten notations, letters, cards, memoranda, diaries, electronic mail, drawings, photographs, audio, DVD and videotape recordings, statements, manuals, calendars, notes of telephone conversations, reports, receipts, correspondence, notes, computer print outs, tapes, disks, CD-ROM, and other forms of electronically or magnetically maintained information. The term "e-mail accounts" includes all email accounts, whether for personal use, business, or otherwise.

3. The terms "relating to" and "related to" mean in whole or in part or in any way constituting, containing, concerning, embodying, evidencing, reflecting, describing, analyzing, identifying, stating, dealing with, referring to or pertaining to.

4. Words used in the singular shall, where the context permits, include the plural, and words used in the plural shall, where the context permits, include the singular.

5. "You" and "your" refers to the person served with and responding to this subpoena.

6. The term "Working Group 112" shall refer to the 18 members who comprised the working group for the International Agency for Research on Cancer ("IARC")'s monograph volume 112: "Some Organophosphate Insecticides and Herbicides: Diazinon, Glyphosate, Malathion, Parathion, and Tetrachlorvinphos" from January 1, 2014 through July 29, 2015; the 17 members who met at IARC on March 3 through March 10, 2015 to assess the carcinogenicity of glyphosate, and worked on IARC monograph 112, as well as invited specialists, observers, representatives of national and international health agencies and IARC secretariats. The individuals who comprise IARC Working Group 112 are identified in Attachment 1 to this document request.

You may provide the following requests either by mail to:

Hollingsworth LLP

1350 I Street, N.W.
Washington, DC 20005
Attn: Kirby Griffis

Or you may choose to contact Kirby Griffis at (202) 898-5828 to arrange a place of inspection/copying/transmittal as convenient to you.

All documents must be provided by no later than **May 1, 2017** at 9:00AM.

DOCUMENT REQUESTS

1. A copy of your most recent curriculum vitae.

2. All documents including without limitation, all emails with any attachments, created by, sent by, received by, copied to, or maintained by you, correspondence, and notes, in your possession that were responsive to Monsanto's subpoena served upon you on or around August 19, 2016 (Attachment 2), that you did not already produce.

UNITED STATES DISTRICT COURT
NORTHERN DISTRICT OF CALIFORNIA

IN RE: ROUNDUP PRODUCTS LIABILITY LITIGATION	MDL No. 2741 Case No. 16-md-02741-VC
This document relates to all cases.	**PLAINTIFFS' CROSS-NOTICE TO TAKE ORAL AND VIDEOTAPED DEPOSITION OF DR. MATTHEW ROSS**

TO: Defendant **MONSANTO COMPANY** by and through its attorney of record Heather Pigman, Hollingsworth LLP, 1350 I Street NW, Washington, DC 20005.

Please take notice that pursuant to Rule 30 of the Federal Rules of Civil Procedure and PTO 16 of MDL 2741, Plaintiffs, by and through their counsel, will take the videotaped deposition upon oral examination of **Matthew K. Ross, Ph.D., on Wednesday, May 3, 2017 at 9:00 a.m. CDT, at Mississippi State University, 175 President's Circle, Allen Hall, Mississippi State, MS 39762**. The witness shall produce documents identified in **Exhibit A**, attached hereto. The deposition will be taken before a person authorized by law to administer oaths, pursuant to Rule 28 of the Federal Rules of Civil Procedure, and will continue day-to-day until the examination is completed. This deposition is cross-noticed in the above-captioned manner pursuant to Federal Rules of Civil Procedure.

DATED: May 2, 2017

By: /s/ Aimee H. Wagstaff

Aimee H. Wagstaff
Andrus Wagstaff, PC
7171 W. Alaska Drive
Lakewood, CO 80226
Tel: 303-376-6360
aimee.wagstaff@andruswagstaff.com
Co-Lead Counsel for Plaintiffs in MDL No. 2741

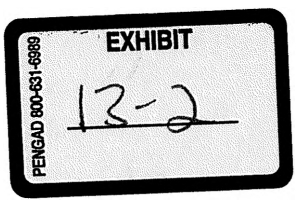


EXHIBIT A

DOCUMENT REQUESTS

Please produce to Noticing Party the following documents at least 48 hours prior to your scheduled deposition:

1. A copy of your most current *Curriculum Vitae*.

CERTIFICATE OF SERVICE

I hereby certify that a true and correct copy of the foregoing document was served on Monsanto via HPigman@Hollingsworthllp.com

DATED: May 2, 2017

By: /s/ Aimee H. Wagstaff

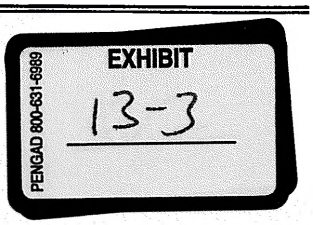
Aimee H. Wagstaff
Andrus Wagstaff, PC
7171 W. Alaska Drive
Lakewood, CO 80226
Tel: 303-376-6360
aimee.wagstaff@andruswagstaff.com
Co-Lead Counsel for Plaintiffs in MDL No. 2741

AO 88B (Rev. 02/14) Subpoena to Produce Documents, Information, or Objects or to Permit Inspection of Premises in a Civil Action

UNITED STATES DISTRICT COURT

for the

Northern District of California



EDWARD HARDEMAN

Plaintiff

v.

MONSANTO COMPANY AND JOHN DOES 1-50

Defendant

Civil Action No. 3:16-cv-00525-VC

SUBPOENA TO PRODUCE DOCUMENTS, INFORMATION, OR OBJECTS OR TO PERMIT INSPECTION OF PREMISES IN A CIVIL ACTION

To: Dr. Matthew K. Ross

(Name of person to whom this subpoena is directed)

☑ *Production:* **YOU ARE COMMANDED** to produce at the time, date, and place set forth below the following documents, electronically stored information, or objects, and to permit inspection, copying, testing, or sampling of the material: See attachment for list of documents to be produced.

Place:	Date and Time:
Place of inspection/copying/transmittal to be arranged with issuing attorney as convenient to Dr. Ross.	09/16/2016 9:00 am

☐ *Inspection of Premises:* **YOU ARE COMMANDED** to permit entry onto the designated premises, land, or other property possessed or controlled by you at the time, date, and location set forth below, so that the requesting party may inspect, measure, survey, photograph, test, or sample the property or any designated object or operation on it.

Place:	Date and Time:

The following provisions of Fed. R. Civ. P. 45 are attached – Rule 45(c), relating to the place of compliance; Rule 45(d), relating to your protection as a person subject to a subpoena; and Rule 45(e) and (g), relating to your duty to respond to this subpoena and the potential consequences of not doing so.

Date: 08/18/2016

CLERK OF COURT

OR

Signature of Clerk or Deputy Clerk

Attorney's signature

The name, address, e-mail address, and telephone number of the attorney representing *(name of party)* Monsanto Company, who issues or requests this subpoena, are:

Eric G. Lasker, 1350 I Street NW, Washington, DC 20005; elasker@hollingsworthllp.com; (202) 898-5800

Notice to the person who issues or requests this subpoena

If this subpoena commands the production of documents, electronically stored information, or tangible things or the inspection of premises before trial, a notice and a copy of the subpoena must be served on each party in this case before it is served on the person to whom it is directed. Fed. R. Civ. P. 45(a)(4).

Civil Action No. 3:16-cv-00525-VC

PROOF OF SERVICE

(This section should not be filed with the court unless required by Fed. R. Civ. P. 45.)

I received this subpoena for *(name of individual and title, if any)* _____

on *(date)* _____ .

❒ I served the subpoena by delivering a copy to the named person as follows: _____

_____

_____ on *(date)* _____ ; or

❒ I returned the subpoena unexecuted because: _____

_____ .

Unless the subpoena was issued on behalf of the United States, or one of its officers or agents, I have also tendered to the witness the fees for one day's attendance, and the mileage allowed by law, in the amount of $ _____ .

My fees are $ _____ for travel and $ _____ for services, for a total of $ 0.00 .

I declare under penalty of perjury that this information is true.

Date: _____

_____
Server's signature

_____
Printed name and title

_____
Server's address

Additional information regarding attempted service, etc.:

Federal Rule of Civil Procedure 45 (c), (d), (e), and (g) (Effective 12/1/13)

(c) Place of Compliance.

(1) ***For a Trial, Hearing, or Deposition.*** A subpoena may command a person to attend a trial, hearing, or deposition only as follows:

(A) within 100 miles of where the person resides, is employed, or regularly transacts business in person; or

(B) within the state where the person resides, is employed, or regularly transacts business in person, if the person

(i) is a party or a party's officer; or

(ii) is commanded to attend a trial and would not incur substantial expense.

(2) ***For Other Discovery.*** A subpoena may command:

(A) production of documents, electronically stored information, or tangible things at a place within 100 miles of where the person resides, is employed, or regularly transacts business in person; and

(B) inspection of premises at the premises to be inspected.

(d) Protecting a Person Subject to a Subpoena; Enforcement.

(1) ***Avoiding Undue Burden or Expense; Sanctions.*** A party or attorney responsible for issuing and serving a subpoena must take reasonable steps to avoid imposing undue burden or expense on a person subject to the subpoena. The court for the district where compliance is required must enforce this duty and impose an appropriate sanction—which may include lost earnings and reasonable attorney's fees—on a party or attorney who fails to comply.

(2) ***Command to Produce Materials or Permit Inspection.***

(A) *Appearance Not Required.* A person commanded to produce documents, electronically stored information, or tangible things, or to permit the inspection of premises, need not appear in person at the place of production or inspection unless also commanded to appear for a deposition, hearing, or trial.

(B) *Objections.* A person commanded to produce documents or tangible things or to permit inspection may serve on the party or attorney designated in the subpoena a written objection to inspecting, copying, testing, or sampling any or all of the materials or to inspecting the premises—or to producing electronically stored information in the form or forms requested. The objection must be served before the earlier of the time specified for compliance or 14 days after the subpoena is served. If an objection is made, the following rules apply:

(i) At any time, on notice to the commanded person, the serving party may move the court for the district where compliance is required for an order compelling production or inspection.

(ii) These acts may be required only as directed in the order, and the order must protect a person who is neither a party nor a party's officer from significant expense resulting from compliance.

(3) ***Quashing or Modifying a Subpoena.***

(A) *When Required.* On timely motion, the court for the district where compliance is required must quash or modify a subpoena that:

(i) fails to allow a reasonable time to comply;

(ii) requires a person to comply beyond the geographical limits specified in Rule 45(c);

(iii) requires disclosure of privileged or other protected matter, if no exception or waiver applies; or

(iv) subjects a person to undue burden.

(B) *When Permitted.* To protect a person subject to or affected by a subpoena, the court for the district where compliance is required may, on motion, quash or modify the subpoena if it requires:

(i) disclosing a trade secret or other confidential research, development, or commercial information; or

(ii) disclosing an unretained expert's opinion or information that does not describe specific occurrences in dispute and results from the expert's study that was not requested by a party.

(C) *Specifying Conditions as an Alternative.* In the circumstances described in Rule 45(d)(3)(B), the court may, instead of quashing or modifying a subpoena, order appearance or production under specified conditions if the serving party:

(i) shows a substantial need for the testimony or material that cannot be otherwise met without undue hardship; and

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(e) Duties in Responding to a Subpoena.

(1) ***Producing Documents or Electronically Stored Information.*** These procedures apply to producing documents or electronically stored information:

(A) *Documents.* A person responding to a subpoena to produce documents must produce them as they are kept in the ordinary course of business or must organize and label them to correspond to the categories in the demand.

(B) *Form for Producing Electronically Stored Information Not Specified.* If a subpoena does not specify a form for producing electronically stored information, the person responding must produce it in a form or forms in which it is ordinarily maintained or in a reasonably usable form or forms.

(C) *Electronically Stored Information Produced in Only One Form.* The person responding need not produce the same electronically stored information in more than one form.

(D) *Inaccessible Electronically Stored Information.* The person responding need not provide discovery of electronically stored information from sources that the person identifies as not reasonably accessible because of undue burden or cost. On motion to compel discovery or for a protective order, the person responding must show that the information is not reasonably accessible because of undue burden or cost. If that showing is made, the court may nonetheless order discovery from such sources if the requesting party shows good cause, considering the limitations of Rule 26(b)(2)(C). The court may specify conditions for the discovery.

(2) ***Claiming Privilege or Protection.***

(A) *Information Withheld.* A person withholding subpoenaed information under a claim that it is privileged or subject to protection as trial-preparation material must:

(i) expressly make the claim; and

(ii) describe the nature of the withheld documents, communications, or tangible things in a manner that, without revealing information itself privileged or protected, will enable the parties to assess the claim.

(B) *Information Produced.* If information produced in response to a subpoena is subject to a claim of privilege or of protection as trial-preparation material, the person making the claim may notify any party that received the information of the claim and the basis for it. After being notified, a party must promptly return, sequester, or destroy the specified information and any copies it has; must not use or disclose the information until the claim is resolved; must take reasonable steps to retrieve the information if the party disclosed it before being notified; and may promptly present the information under seal to the court for the district where compliance is required for a determination of the claim. The person who produced the information must preserve the information until the claim is resolved.

(g) Contempt.

The court for the district where compliance is required—and also, after a motion is transferred, the issuing court—may hold in contempt a person who, having been served, fails without adequate excuse to obey the subpoena or an order related to it.

For access to subpoena materials, see Fed. R. Civ. P. 45(a) Committee Note (2013).

DEFINITIONS AND INSTRUCTIONS

1. The term "Communication," as used in these Requests, is intended to have the broadest possible meaning and shall include any contact or act by which information or knowledge is transmitted or conveyed between two or more persons and includes, without limitation: (1) written contact, including but not limited to letters, memoranda, PowerPoint presentations, email, text message, telegram, telex, internet-based meetings, or other written or electronic documents or files; (2) oral contact, whether by face-to-face meetings, internet-based meetings, video conferences, telephonic conversations, or otherwise; and (3) nonverbal acts intended to communicate or convey any meaning, understanding or other message.

2. The term "documents" is used broadly, and encompasses all tangible things and recorded information possessed by you, whether such documents are located in computers, e-mail accounts, or hard-copy documents or files. The term "documents" includes, but is not limited to, handwritten, typed, or printed papers, whether in final or draft form, handwritten notations, letters, cards, memoranda, diaries, electronic mail, drawings, photographs, audio, DVD and videotape recordings, statements, manuals, calendars, notes of telephone conversations, reports, receipts, correspondence, notes, computer print outs, tapes, disks, CD-ROM, and other forms of electronically or magnetically maintained information.

3. The terms "relating to" and "related to" mean in whole or in part or in any way constituting, containing, concerning, embodying, evidencing, reflecting, describing, analyzing, identifying, stating, dealing with, referring to or pertaining to.

4. Words used in the singular shall, where the context permits, include the plural, and words used in the plural shall, where the context permits, include the singular.

5. "You" and "your" refers to the person served with and responding to this subpoena.

6. The term "IARC Working Group 112" shall refer to the 18 members who comprised the working group for the International Agency for Research on Cancer ("IARC")'s monograph volume 112: "Some Organophosphate Insecticides and Herbicides: Diazinon, Glyphosate, Malathion, Parathion, and Tetrachlorvinphos" from January 1, 2014 through July 29, 2015; the 17 members who met at IARC on March 3 through March 10, 2015 to assess the carcinogenicity of glyphosate, and worked on IARC monograph 112, as well as invited specialists, observers, representatives of national and international health agencies and IARC secretariats. The individuals who comprise IARC Working Group 112 are identified in Attachment 1 to this document request.

7. The term "other organizations and individuals" shall include, but is not limited to, the following individuals and non-governmental entities: Greenpeace, the Natural Resources Defense Council, Waterkeeper Alliance, Slow Food USA, Earth Eats, AVAAZ, Environmental Defense Fund, Occupy Wall Street, Environmental Working Group, EcoWatch, Food Democracy Now!, Just Label it!, GMO Free USA, Center 4 Food

Safety, Alex Jones, Rob Schneider, Norman Buffong, Randall Grahm, and Dr. Joseph Mercola.

You may provide the following requests either by mail to:

Hollingsworth LLP
1350 I Street, N.W.
Washington, DC 20005
Attn: Neil Bromberg

Or you may choose to contact Neil Bromberg at (202) 898-5805 to arrange a place of inspection/copying/transmittal as convenient to you.

All documents must be provided by no later than **September 16, 2016** at 9:00AM.

DOCUMENT REQUESTS

1. All documents, including all emails with any attachments, created by, sent by, received by, copied to, or maintained by you relating to or referring to the International Agency for Research on Cancer ("IARC") Working Group 112.

2. All communications, including without limitation, emails, correspondence, notes, and other documents exchanged between you and any member of IARC Working Group 112, or anyone attending meetings of IARC Working Group 112, regarding glyphosate.

3. All drafts of Monograph 112 on glyphosate, including drafts of individual sections of Monograph 112, whether written by you or anyone else.

4. All research, studies, analyses, calculations, re-evaluations of previously published studies, or data you reviewed, drafted, generated, or received in connection with IARC Working Group 112.

5. All notes, writings, and recordings (whether by audio or visual means) taken during any meeting of, or communications with, IARC Working Group 112 members, whether in person, over the telephone, or over the Internet. This request should be read broadly to include meetings or communications with individual IARC Working Group 112 members, or smaller subgroups of IARC Working Group 112 members.

6. All documents, including all emails with any attachments, created by, sent by, received by, copied to, or maintained by you relating to or referring to IARC generally.

7. All documents, including all emails with any attachments, created by, sent by, received by, copied to, or maintained by you relating to or referring to glyphosate, glyphosate containing-herbicides (including, but not limited to, Roundup-branded herbicides), or aminomethylphosphonic acid ("AMPA").

8. All documents, including all emails with any attachments, created by, sent by, received by, copied to, or maintained by you relating to or referring to Monsanto and/or any other manufacturer of glyphosate-based herbicides.

9. All communications, including without limitation, emails, correspondence, notes, and other documents exchanged between you and the United States Environmental Protection Agency, or any other federal, state or local government agency, relating to or referring to glyphosate, glyphosate containing-herbicides (including, but not limited to, Roundup-branded herbicides), AMPA, Monsanto, any other manufacturer of glyphosate-based herbicides, or IARC.

10. All communications, including without limitation, emails, correspondence, notes, and other documents exchanged between you and any agency of a foreign government, or any non-governmental agency, including the European Union, relating to or referring to glyphosate, glyphosate containing-herbicides (including, but not limited to, Roundup-branded herbicides), AMPA, Monsanto, any other manufacturer of glyphosate-based herbicides, or IARC.

11. All documents relating to any review, re-analysis, or statistical calculations, you performed, reviewed, commented on, or in any way contributed to on previously published or unpublished studies, including animal studies, or other data in connection with IARC Working Group 112.

12. All documents relating to the trend analysis calculations you or others did that are referenced at page 33 of the IARC Working Group 112 monograph on glyphosate.

13. All documents, including all emails with attachments, created by, sent by, received by, copied to, or maintained by you regarding the review by you or others of the specific microscopic evidence and histologic evaluation of the 1983 mouse study referenced in studies at page 33 of the IARC Working Group 112 monograph on glyphosate (appended hereto as Attachment 2).

14. All conflict of interest statements, declaration of interest statements, or other documents, emails or forms referencing any potential conflict of interest, that you sent or submitted to, or received from, any United States federal, state or local agency, IARC, or any agency of a foreign government, including the European Union, regarding any potential conflict of interest you might have in working for, advising, consulting with, or performing any task for these agencies and governments.

15. All communications with attorneys, law firms, or other individuals anywhere in the world who have brought or intend to bring lawsuits against Monsanto, and/or any other manufacturer of glyphosate-based herbicides, including without limitation, emails, correspondence, notes, and other documents that were exchanged.

16. All communications, including without limitation, emails, correspondence, notes, and other documents relating to or referring to glyphosate, glyphosate-containing herbicides (including, but not limited to, Roundup-branded herbicides), AMPA, Monsanto and/or

any other manufacturer of glyphosate-based herbicides, or IARC, that were exchanged between you and the other organizations and individuals identified in Definition No. 7.

17. All communications, including without limitation, emails, correspondence, notes, and other documents, exchanged after the publication of IARC Working Group 112 monograph between you and any member of IARC Working Group 112, the United States Environmental Protection Agency, any other federal, state or local government agency, or any agency of a foreign government including the European Union, relating to or referring to glyphosate, glyphosate-containing herbicides (including, but not limited to, Roundup-branded herbicides), AMPA, Monsanto, any other manufacturer of glyphosate-based herbicides, or IARC.

18. All documents regarding any trips, visits, or contact made (whether in person, over the telephone, or internet) with the United States Environmental Protection Agency, any other federal, state or local government agency, or any agency of a foreign government including the European Union and the World Health Organization, regarding glyphosate, glyphosate-containing herbicides (including, but not limited to, Roundup-branded herbicides), AMPA, Monsanto, any other manufacturer of glyphosate-based herbicides, other pesticides, genetically modified food, or IARC.

19. All communications, including without limitation, emails, correspondence, notes, and other documents created by, sent by, received by, copied to, or maintained by you, relating to speaking engagements, presentations, hearings, or conferences which you have attended, presented on or spoken on, relating to or referring to glyphosate, glyphosate-containing herbicides (including, but not limited to Roundup-branded herbicides), AMPA, Monsanto, any other manufacturer of glyphosate-based herbicides, or IARC.

20. All documents, studies, letters to the editor, interviews and/or articles you have published or submitted for publication or any kind of peer review on glyphosate, glyphosate-containing herbicides (including, but not limited to Roundup-branded herbicides), AMPA, Monsanto, any other manufacturer of glyphosate-based herbicides, or IARC.

21. All documents, including without limitation, emails, correspondence, communications, commentary, notes, and other documents created by, sent by, received by, copied to, or maintained by you, relating to (a) Christopher Portier's Open letter: Review of the Carcinogenicity of Glyphosate by EFSA and BfR to Commissioner Andriukaitis (Nov. 27, 2015) (appended as Attachment 3) and (b) Christopher J. Portier, *et al.*, Differences in the carcinogenic evaluation of glyphosate between the International Agency for Research on Cancer (IARC) and the European Food Safety Authority (EFSA), J Epidemiol Community Health Month (Mar. 2016) (appended as Attachment 4).

22. All documents, including without limitation, emails, correspondence, communications, commentary, notes, and other documents created by, sent by, received by, copied to, or maintained by you relating to or referring to surfactants used in glyphosate-based herbicides, including the group of surfactants known as polyethoxylated tallow amine ("POEAs").

Updated: May 2017

CURRICULUM VITAE

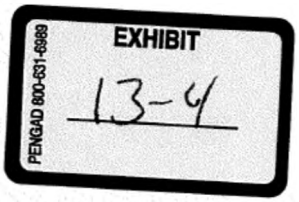
Matthew K. Ross, Ph.D.
Mississippi State University
Department of Basic Sciences
Center for Environmental Health Sciences
College of Veterinary Medicine
[redacted]

EDUCATION

1998	**Ph.D., Molecular Toxicology** University of California at Irvine
1989	**B.S., Chemistry** University of California at Berkeley

RESEARCH AND PROFESSIONAL EXPERIENCE

08/10-Present	**Associate Professor,** Mississippi State University (Awarded tenure, July 2010) Department of Basic Sciences Center for Environmental Health Sciences College of Veterinary Medicine
01/04-07/10	**Assistant Professor,** Mississippi State University Department of Basic Sciences Center for Environmental Health Sciences College of Veterinary Medicine
10/99–12/03	**Postdoctoral Fellow** Curriculum in Toxicology University of North Carolina, Chapel Hill
2/98–9/99	**Postdoctoral Fellow** Dept. of Community & Environmental Medicine School of Medicine University of California, Irvine
9/92–2/98	**Research Assistant** Dept. of Community & Environmental Medicine Environmental Toxicology Graduate Program School of Medicine University of California, Irvine
7/89–8/92	**Research Chemist/Group Leader** Plant/Soil Metabolism Group PTRL-West, Richmond, CA
1987–1989	**Chemistry Stockroom/Teaching Assistant** College of Chemistry University of California, Berkeley



AWARDS/HONORS RECEIVED

2015	Visiting Foreign Scientist, *Jiangsu Academy of Agricultural Sciences* (JAAS), June 1-30 2015, Nanjing, China
2015	Invited Working Group Member, *International Agency for Research on Cancer* (IARC), March 2016, Lyon, France
2012	*Honorary Professor*, *Jiangsu Academy of Agricultural Sciences* (JAAS) Nanjing, China
2011	*Mississippi Veterinary Medical Association (MVMA) Faculty Award*, MSU
2010	*Richard C. Adkerson Faculty Award,* MSU
2008	*Pegasus Dean's Research Award*, College of Veterinary Medicine, MSU
2008	*Pfizer Animal Health Research Award*, College of Veterinary Medicine, MSU
2008	*College of Veterinary Medicine Faculty Research Award*, Office of Research and Economic Development, MSU
2001-2003	*National Research Service Award* (NRSA) from NIH (Postdoctoral fellowship, F32 ES11111)
1997-1998	*UC Irvine Dissertation Fellowship,* University of California at Irvine
1997	*UC Irvine Cancer Center Travel Award*, University of California at Irvine
1994	*Society of Toxicology Travel Award*, University of California at Irvine
1986	*Saddleback College Chemistry Scholarship* to obtain Chemistry B.S. at U.C. Berkeley ($15,000)

PROFESSIONAL SOCIETIES

American Chemical Society (ACS)
International Society for the Study of Xenobiotics (ISSX)
Society of Toxicology (SOT)

RESEARCH (FTE 70%)

PEER-REVIEWED PUBLICATIONS

Publications since joining MSU in 2004:

Jung Hwa Lee, Evangel Kummari, Abdolsamad Borazjani, Mariola J. Edelmann, and **Matthew K. Ross** (2017) Characterization of Serine Hydrolases and Altered Endocannabinoid Metabolism in Chicken Macrophages (HD11) Following Infection with *Salmonella enterica* serovar Typhimurium. In preparation.

Lee C. Mangum, Abdolsamad Borazjani, Jung Hwa Lee, Xiang Hou, **Matthew K. Ross***, and J. Allen Crow* (2017) Silencing Carboxylesterase 1 in THP-1 Macrophages Affects the Transcription of Cholesterol Metabolism Genes. Under revision at *BBA Molecular and Cell Biology of Lipids*. *Both authors contributed equally.

Kristen M. Fizzano, Andrew K. Claude, Lan-Hsin Kuo, Jeffrey B. Eells, Simone B. Hinz, Brittany E. Thames, Matthew K. Ross, Robert L. Linford, Robert W. Wills, Alicia K. Olivier, Todd M. Archer (2017) Evaluation of a modified maxillary nerve block for canine rhinoscopy with nasal biopsy. *American Journal of Veterinary Research*. Pending revisions.

64. Muro S., Lee J.H., Stokes J., **Ross M.K.**, Archer T.M., Wills R.W., Mackin A.J., and Thomason J.M. (2017) Effects of Leukoreduction and Storage on Erythrocyte Phosphatidylserine Expression and Eicosanoid Levels in Units of Canine Packed Red Blood Cells. *J. Vet. Intern. Med.* **31**, 410-418.

63. Matthews A.T.*, Lee J.H.*, Borazjani A., Mangum L.C., Hou X., **Ross M.K.** (2016) Oxyradical Stress Increases the Biosynthesis of 2-Arachidonoylglycerol: involvement of NADPH Oxidase. *Am. J. Physiol. Cell Physiol.* **311**, C960-C974. *These authors contributed equally to this work.

62. Chambers, J.E., Chambers, H.W., Funck, K.W., Meek, E.C., Pringle, R.B., and **Ross, M.K.** (2016) Efficacy of Novel Phenoxyalkyl Pyridinium Oximes as Brain-Penetrating Reactivators of Cholinesterase Inhibited by Surrogates of Sarin and VX. *Chemico-Biol. Interact*. **259** (Pt B), 154-159.

61. Portier, C.J. et al. (Ross, M.K. was one of 93 co-authors) (2016) Differences on the carcinogenicity of glyphosate between the International Agency for Research on Cancer (IARC) and the European Food Safety Agency (EFSA). *J. Epidemiol. Community Health*. **70**, 741-745.

60. Mangum, L.C., Mangum, L.H., Chambers, J.E., **Ross, M.K.**, Meek, E.C., Wills, R.W., and Crow, J.A. (2016) Serum levels of the organochlorine trans-nonachlor, but not urinary isoprostanes, improves the ability of a multivariable regression model to predict atherosclerosis outcomes. *J. Toxicol. Environ. Health, Part A.* **8**, 1-11.

59. Mangum, L.H., Crow, J.A., Stokes, J.V., Howell III, G.E., **Ross**, **M.K.**, Pruett, S.B., Chambers, J.E. (2016) Exposure to p,p'-DDE alters macrophage reactivity and increases macrophage numbers in adipose stromal vascular fraction. *Toxicol. Sci.* **150**, 169-177.

58. Carr, R.L., Armstrong, N.H., Buchanan, A.T., Eells, J.B., Mohammed, A.N., **Ross, M.K.**, Nail, C.A. (2015) Altered Emotional Reactivity in Rats Following Exposure to Low Levels of Chlorpyrifos During Development. *Neurotoxicology*. **In press.**

57. Matthews, A.T. and **Ross, M.K.** (2015) Oxyradical stress, endocannabinoids, and atherosclerosis. *Toxics.* **3**, 481-498.

56. **Ross, M.K.**, Pluta, K., Bittles, V., Borazjani, A., Crow, J.A. (2016) Interactions of the Serine Hydrolase KIAA1363 with Organophosphorus Agents: Evaluation of Potency and Kinetics. *Arch. Biochem. Biophys.* **590**, 72-81.

55. Szafran, B., Borazjani A., Lee, J.H., **Ross, M.K.**, Kaplan, B.L.F. (2015) Lipopolysaccharide Suppresses Carboxylesterase 2g Activity and 2-Arachidonylglycerol Hydrolysis: A Possible Mechanism to Regulate Inflammation. *Prostaglandins and Other Lipid Mediators*. **121**, 199-206.

54. Blake, R.R., Lee, J.H., **Ross, M.K.**, Archer, T.M., Wills, R.W., Mackin, A.J., Thomason, J.M. (2017) Evaluation of eicosanoid concentrations in stored units of canine packed red blood cells. *J. Am. Vet. Med. Assoc.* **250**, 191-198.

53. **Ross, M.K.** and Wang, R. (2015) Expanding the toolkit for the serine hydrolases. *Chemistry & Biology* **22**, 808-809.

52. Guyton, K.Z., Loomis, D., Grosse, Y., Guha, N., Benbrahim-Tallaa, L., El Ghissassi, F., Scoccianti, C., Mattock, H., Straif, K., on behalf of the International Agency for Research on Cancer (IARC) Monograph Working Group (2015) Carcinogenicity of Tetrachlorvinphos, Parathion, Malathion, Diazinon and Glyphosate. *The Lancet Oncology* **16**, 490-491. *Role*: Member of IARC Monograph Working Group.

51. Mangum, L.C., Borazjani, A., Stokes, J.V., Matthews, A.T., Lee, J.H., Chambers, J.E., **Ross, M.K.** (2015) Organochlorine Insecticides Induce NADPH Oxidase-Dependent Reactive Oxygen Species in Human Monocytic Cells via Phospholipase A2/Arachidonic Acid. *Chem. Res. Toxicol.* **28**, 570-584.

50. Chiavaccini, L., Claude, A.K., Lee, J.H., **Ross, M.K.**, Meyer, R.E., Langston, V.C. (2015) Pharmacokinetics and pharmacodynamics comparison between subcutaneous and intravenous butorphanol administration in horses. *Journal of Veterinary Pharmacology and Experimental Therapeutics* **38**, 365-374.

49. **Ross, M.K.**, Borazjani, A., Mangum, L.C., Wang, R., Crow, J.A. (2014) Effects of Toxicologically Relevant Xenobiotics and the Lipid-Derived Electrophile 4-Hydroxynonenal on Macrophage Cholesterol Efflux: Silencing Carboxylesterase 1 Has Paradoxical Effects on Cholesterol Uptake and Efflux. *Chem. Res. Toxicol.* **27**, 1743-1756.

48. **Ross, M.K.**, Matthews, A.T., Mangum, L.C. (2014) Chemical Atherogenesis: Role of Endogenous and Exogenous Poisons in Disease Development. *Toxics* **2**, 17-34.

47. Claude, A.K., Miller W.W., Beyer, A.M., Willeford, K.O., **Ross, M.K.** (2014) Quantification and comparison of baseline cortisol levels between aqueous and plasma from healthy anesthetized hound dogs utilizing mass spectrometry. *Veterinary Ophthalmology* **17**, 57-62.

46. Haraschak J.L., Langston V.C., Wang R., Riggs C., Fellman C., **Ross M.K.**, Bulla C., Lunsford K., Mackin A., Archer T. (2014) Pharmacokinetic Evaluation of Oral Dantrolene in the Dog. *Journal of Veterinary Pharmacology and Experimental Therapeutics* **37**, 286-294.

45. Carr, R.L., Graves, C.A., Mangum, L.C., Nail, C.A., and **Ross, M.K.** (2014) Low Level Chlorpyrifos Exposure Increases Anandamide Accumulation in Juvenile Rat Brain in the Absence of Cholinesterase Inhibition. *Neurotoxicology* **43**, 82-89.

44. Wang, R., Borazjani, A., Matthews, A.T., Mangum, L.C., Edelmann, M.E., **Ross, M.K.** (2013) Identification of palmitoyl protein thioesterase 1 in human THP-1 monocytes/macrophages and characterization of unique biochemical activities for this enzyme. *Biochemistry* **52**, 7559–7574.

43. Ammari, M.G., Pharr, G.T., **Ross, M.K.,** Pinchuk, G.V., Pinchuk L.M. (2013) Mitochondrial dysfunction associated with viral cytopathogenicity. *Current Topics in Virology* **11**, 19-30.

42. Lin, Z., Fisher, J.W., Wang, R., **Ross, M.K.**, Filipov, N.M. (2013) Estimation of placental and lactational transfer and tissue distribution of atrazine and its main metabolites in the rat dam, fetus, and neonate with physiologically based pharmacokinetic modeling. *Toxicol. Appl. Pharmacol.* **273**, 140-158.

41. Carr, R.L., Adams A.L., Kepler D.R., Ward A.B., and **Ross, M.K.** (2013) Induction of Endocannabinoid Levels in Juvenile Rat Brain Following Developmental Chlorpyrifos Exposure. *Toxicol. Sci.* **135**, 193-201.

40. Alavanja, M.C.R., **Ross, M.K.**, Bonner, M.R. (2013) *Reply to*: Increased cancer burden among pesticide applicators and others due to pesticide exposure. *CA: A Cancer Journal for Clinicians*. **63**, 366-367.

39. Alavanja, M.C.R., **Ross, M.K.**, Bonner, M.R. (2013) Increased cancer burden among pesticide applicators and others due to pesticide exposure. *CA: A Cancer Journal for Clinicians*. **63**, 120-142.

38. Figueiredo, A.S., García-Crescioni, H.J., Bulla, S.C., **Ross, M.K.**, McIntosh, C., Lunsford, K., Bulla, C. (2013) Cannabinoid suppression of vascular endothelial growth factor expression in a canine osteosarcoma cell line. *Veterinary Medicine: Research and Reports* **4,** 1-4.

37. Crow, J.A., Bittles, V., Borazjani, A., Potter, P.M., and **Ross, M.K.** (2012) Covalent Inhibition of Recombinant Human Carboxylesterase 1 and 2 and Monoacylglycerol Lipase by the Carbamates JZL184 and URB597. *Biochem. Pharmacol.* **84**, 1215-1222.

36. **Ross, M.K.**, Borazjani, A., Wang, R., Crow, J.A., Xie, S. (2012) Examination of the carboxylesterase phenotype in human liver. *Arch. Biochem. Biophys*. **522**, 44-56.

35. **Ross, M.K.** and Edelmann, M.J. (2012) Carboxylesterases: A Multifunctional Enzyme Involved in Pesticide and Lipid Metabolism. *American Chemical Society (ACS) Symposium Series*. In: Parameters for Pesticide QSAR and PBPK/PD Models, Chapter 10, 149-164.

34. Meek E.C., Chambers H.W., Coban A., Funck K.E., Pringle R.B., **Ross M.K.**, Chambers J.E. (2012) Synthesis and *In Vitro* and *In Vivo* Inhibition Potencies of Highly Relevant Nerve Agent Surrogates. *Toxicol. Sci.* **126**, 525-533.

33. Crow J.A., Bittles V., Herring K.L., Borazjani A., Potter P.M., and **Ross M.K.** (2012) Inhibition of Recombinant Human Carboxylesterase 1 and 2 and Monoacylglycerol Lipase by Chlorpyrifos Oxon, Paraoxon and Methyl Paraoxon. *Toxicol. Appl. Pharmacol.* **258**, 145–150.

32. Lenarduzzi T., Langston C., and **Ross, M.K.** (2011) Pharmacokinetics of Clindamycin-HCl Administered Orally to Pigeons. *J. Avian Med. Surg.* **25**, 259-265.

31. Borazjani A., Edelmann M.J., Hardin K.L., Herring K.L., Crow J.A., and **Ross M.K.** (2011) Catabolism of 4-Hydroxy-2-*trans*-Nonenal by THP1 Monocytes/Macrophages and Inactivation of Carboxylesterases by this Lipid Electrophile. *Chemico-Biol. Interact.* **194**, 1-12.

30. Carr R.L., Borazjani A., and **Ross M.K.** (2011) Effect of Developmental Chlorpyrifos Exposure on Endocannabinoid Metabolizing Enzymes in the Brain of Juvenile Rats. *Toxicol. Sci.* **122**, 112-120.

29. Lin Z., Fisher J.W., **Ross M.K.**, Filipov N.M. (2011) A Physiologically Based Pharmacokinetic Model for Atrazine and its Main Metabolites in the Adult Male C57BL/6 Mouse. *Toxicol. Appl. Pharmacol.* **251**, 16-31.

28. Xie S., Borazjani A., Hatfield M.J., Edwards C.C., Potter P.M., and **Ross M.K.** (2010) Inactivation of lipid glyceryl ester metabolism in human THP1 monocytes/macrophages by activated organophosphorus insecticides: Role of carboxylesterase 1 and 2. *Chem. Res. Toxicol.* **23**, 1890-1904.

27. **Ross M.K.**, Streit T.M., Herring K.L., Xie S. (2010) Carboxylesterases: Dual roles in lipid and pesticide metabolism. *J. Pest. Sci.* **35**, 257-264.

26. Crow J.A., Herring K.L., Xie S., Borazjani A., Potter P.M., and **Ross M.K.** (2010) Inhibition of carboxylesterase activity of THP1 monocytes/macrophages and recombinant human carboxylesterase 1 by oxysterols and fatty acids. *Biochim. Biophys. Acta* (*Molecular and Cell Biology of Lipids*) **1801**, 31-41.

25. Coyne C., **Ross M.K.**, Bailey J. (2009) Dual potency of anti-HER2/neu and anti-EGFR anthracycline-immunoconjugates in chemotherapeutic-resistant mammary carcinoma combined with cyclosporine A and verapamil P-glycoprotein inhibition. *J. Drug Target.* **17,** 474-489.

24. **Ross M.K.**, Jones T., Filipov N.M. (2009) Disposition of the herbicide 2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine (atrazine) and its major metabolites in mice: a liquid chromatography/mass spectrometry analysis of urine, plasma, and tissue levels. *Drug Metab.Dispos.* **37,** 776-786.

23. Crow J.A., Middleton B.L., Borazjani A., Hatfield M.J., Potter P.M., and **Ross M.K.** (2008) Inhibition of carboxylesterase 1 is associated with cholesteryl ester retention in human THP-1 monocyte/macrophages. *Biochim. Biophys. Acta* (*Molecular and Cell Biology of Lipids*) **1781,** 643-654.

22. Das P.C., Streit T.M., Cao Y., Rose R.L., Cherrington N., **Ross M.K.**, Wallace A.D., Hodgson E. (2008) Pyrethroids: cytotoxicity and induction of CYP isoforms in human hepatocytes. *Drug Metab. Drug Interact.* **23,** 211-236.

21. Streit T.M., Borazjani A., Lentz S., Wierdl M., Potter P.M., Gwaltney S.R., and **Ross M.K.** (2008) Evaluation of the 'side-door' in carboxylesterase-mediated catalysis and inhibition. *Biol. Chem.* **389,** 149-162.

20. **Ross M.K.** and Crow J.A. (2007) Role of carboxylesterases in xenobiotic and endobiotic metabolism. *J. Biochem. Mol. Toxicol.* **21,** 187-196.

19. Godin S.J., Crow, J.A., Scollon E.J., Hughes M.F., DeVito M.J., and **Ross M.K.** (2007) Identification of rat and human cytochrome P450 isoforms and a rat serum esterase that metabolize the pyrethroid insecticides deltamethrin and esfenvalerate. *Drug Metab. Dispos.* **35,** 1664-1671.

18. Crow J.A., Borazjani A., Potter P.M., and **Ross M.K.** (2007) Hydrolysis of pyrethroids by human and rat tissues: Examination of intestinal, liver and serum carboxylesterases. *Toxicol. Appl. Pharmacol.* **221,** 1-12.

17. **Ross M.K.** and Borazjani A. (2007) Unit 14.24: Enzymatic activity of human carboxylesterases. *Curr. Protocol. Toxicol.* 4.24.1-4.24.14.

16. Sistrunk S., **Ross M.K.**, Filipov N.M. (2007) Direct effects of manganese compounds on dopamine and its metabolite DOPAC: An in vitro study. *Environ. Toxicol. Pharmacol.* **23,** 286-296.

15. Godin S.J., Scollon E.J., Hughes M.F., Potter P.M., DeVito M.J., and **Ross M.K.** (2006) Species differences in the in vitro metabolism of deltamethrin and esfenvalerate: Differential oxidative and hydrolytic metabolism by humans and rats. *Drug Metab. Dispos.* **34,** 1764-1771.

14. **Ross M.K.** and Filipov N.M. (2006) Determination of atrazine and its metabolites in mouse urine and plasma by LC-MS analysis. *Anal. Biochem.* **351,** 161-173.

13. **Ross M.K.**, Borazjani A., Edwards C.C., Potter P.M. (2006) Hydrolytic metabolism of pyrethroids by human and other mammalian carboxylesterases. *Biochem. Pharmacol.* **71,** 657-669.

12. Granville C.*, **Ross M.K.***, Tornero-Velez R., Hanley N., Grindstaff R., Gold A., Richard A., Funasaka K., Evans M.V., DeMarini D.M. (2005) Genotoxicity and metabolism of the source water contaminant 1,1-dichloropropene: Activation by *GSTT1-1*. *Mutat. Res.* **572,** 98-112.
* *Both authors contributed equally to this work.* (This manuscript was written in part while setting up my laboratory at MSU; the experimental work was completed while I was a postdoc)

Publications from postdoctoral and graduate work:

11. **Ross M.K.** and Pegram R.A. (2004) *In vitro* biotransformation and genotoxicity of the drinking water disinfection byproduct bromodichloromethane: DNA binding mediated by glutathione transferase theta 1-1. *Toxicol. Appl. Pharmacol.* **195,** 166-181.

10. Geter D.R., Chang L.W., Hanley N.M., **Ross M.K.**, Pegram R.A., DeAngelo A.B. (2004) Analysis of in vivo and in vitro DNA strand breaks from trihalomethane exposure. *J. Carcinogenesis* **3,** 2.

9. Tornero-Velez R., **Ross M.K.**, Granville C., Laskey J., Jones J.P., DeMarini D.M., Evans M.V. (2004) Metabolism and mutagenicity of source water contaminants 1,3-dichloropropane and 2,2-dichloropropane. *Drug Metab. Dispos.* **32,** 123-131.

8. **Ross M.K.** and Pegram R.A. (2003) Glutathione transferase theta 1–1-dependent metabolism of the water disinfection byproduct bromodichloromethane. *Chem. Res. Toxicol.* **16**, 216-226.

7. **Ross M.K.** and Pegram R.A. (2003) [^{35}S]-Labeling of the *Salmonella typhimurium* glutathione pool to assess glutathione-mediated DNA binding by 1,2-dibromoethane. *Chem-Biol. Interact.* **146**, 39-49.

6. Landi S., Naccarati A., **Ross M.K.**, Hanley N.M., Daley L., Devlin R., Vasquez M., Pegram R.A., DeMarini D.M. (2003) Induction of DNA strand breaks by trihalomethanes in primary human lung epithelial cells. *Mutat. Res.* **538**, 41-50.

5. **Ross M.K.**, Said B., Shank R.C. (2000) DNA-damaging effects of genotoxins in mixture: Modulation of covalent binding to DNA. *Toxicol. Sci.* **53**, 224-236.

4. Said B., **Ross M.K.**, Hamade A.K., Matsumoto D.C., Shank R.C. (1999) DNA-damaging effects of genotoxins in mixture: Nonadditive effects of aflatoxin B_1 and *N*-acetylaminofluorene on their mutagenicity in *Salmonella typhimurium*. *Toxicol. Sci.* **52**, 226-231.

3. **Ross M.K.**, Mathison B.M., Said B., Shank R.C. (1999) 5-Methylcytosine in CpG sites and the reactivity of nearest neighboring guanines towards the carcinogen aflatoxin B_1-8,9-epoxide. *Biochem.Biophys.Res.Comm.* **254**, 114-119.

2. **Ross M.K.** (1998) DNA-damaging effects of genotoxins in mixture: Modulation of covalent binding to DNA. Ph.D. Dissertation. University of California at Irvine.

1. Said B., **Ross M.K.**, Salib T., Shank R.C. (1995) Modulation of DNA adduct formation by successive exposures of DNA to small and bulky chemical carcinogens. *Carcinogenesis* **16**, 3057-3062.

BOOK CHAPTERS/MONOGRAPHS

IARC (2016) IARC Monographs Programme: Pentachlorophenol and Some Related Compounds. Vol. 117. (http://monographs.iarc.fr/ENG/Monographs/vol117/index.php) – working group member

IARC (2015) IARC Monographs Programme: Some Organophosphate Insecticides and Herbicides: Diazinon, Glyphosate, Malathion, Parathion, and Tetrachlorvinphos. Vol. 112. (http://monographs.iarc.fr/ENG/Monographs/vol112/index.php) – working group member

Ross M.K. (2011) The Pyrethroid Insecticides. In: *Encyclopedia of Environmental Health.* volume 4, pp. 702–708, Elsevier Ltd., Oxford, UK, *Ed.* Jerome Nriagu. (Invited book chapter).

Chambers J.E., Meek E.C., **Ross M.K.** (2010) The Metabolic Activation and Detoxication of Anticholinesterase Insecticides. In: *Anticholinesterase Pesticides: Metabolism, Neurotoxicity, and Epidemiology*, chapter 6, pp. 77-84, Wiley, New York, *Ed.* Ramesh Gupta and Tetsuo Satoh. (Invited book chapter).

CURRENT RESEARCH SUPPORT

Mississippi Food Safety Initiative Ross (PI) 05/01/14-06/30/17 ($40,000)
Sponsor: USDA

Title: Targeting the Endocannabinoid System to Enhance Immunity

Goals: The goal of this project will be the identification of serine hydrolases in macrophages that can be targeted (i.e. inhibited) by small molecules for the purpose of enhancing endocannabinoid levels during microbial infection, and whether the microbicidal activity of the macrophages is concomitantly enhanced.

Role: Principal Investigator

Responsibilities: Overall management of project, design and perform experiments, write annual reports, and manuscript writing.

1R15ES015348-02 **Ross (PI)** **02/08/12-01/31/17** **($425,457)**
Sponsor: NIH

Title: Lipid Glyceryl Ester Homeostasis in Macrophages and Perturbation by Environmental Toxicants

Goals: This project examines the mechanisms by which endogenous toxins (oxidized low density lipoproteins) and exogenous toxicants (pesticides) can together dysregulate the endocannabinoid system in macrophages, thus enhancing foam cell development.

Role: Principal Investigator

Responsibilities: Overall management of project, design and perform experiments, write annual reports, and manuscript writing.

1R15GM116129-01 **Crow (PI)** **07/01/15-06/30/18** **($425,457)**
Sponsor: NIH

Title: Discovery of endogenous pro-ligands regulated by CES1

Goals: This project will characterize the endogenous substrates for CES1 that are pro-ligands for the lipid sensor/nuclear receptor PPAR gamma.

Role: Co-Investigator (M.K. Ross)

Responsibilities: Management of aim 2 and part of aim 3, design and perform experiments, help to write annual reports, and perform manuscript writing.

1R15ES023162-01A1 **Carr (PI)** **12/01/14-11/30/17** **($426,959)**
Sponsor: NIH

Title: Disruption of the Endocannbinoid System as a Target in Developmental OP Toxicity

Goals: This project examines the endocannabinoid system as a target of developmental OP toxicity.

Role: Co-Investigator (M.K. Ross)

Responsibilities: LC-MS/MS metabolipidomic analysis of 2-arachidonoylglycerol, anandamide and other bioactive lipids.

D15CA-805 **Thomason (PI)** **08/01/14-07/31/15** **($10,697)**
Sponsor: Morris Animal Foundation

Title: Effects of Leukoreduction on Eicosanoid Biosynthesis in Stored Canine Packed Red Blood Cells.

Goals: This project examines whether storage of canine packed red cells leads to the increased production of bioactive eicosanoids.

Role: Co-Investigator (M.K. Ross)

Responsibilities: Oversee the analysis of eicosanoids by LC-MS/MS.

F31 HL122082-02 Matthews (PI) 08/15/14-08/14/16
Sponsor: NIH

Title: Role of endocannabinoids in atherosclerosis.

Goals: This is a pre-doctoral fellowship to study whether endocannabinoid biosynthesis is enhanced following ligation of the macrophage scavenger receptor CD36 by oxidized low-density lipoprotein as part of a compensatory mechanism to counteract inflammation and oxidative stress. Specifically, this project will determine whether diacylglycerol lipase β (DAGLβ), the rate-limiting biosynthetic enzyme of 2-AG, is activated via transduction of Nox-derived reactive oxygen species.

Role: Co-mentors (M.K. Ross; Stephen Pruett)

Responsibilities: Oversee the training and mentorship of PhD student Anberitha Matthews

Grant: EPA Star Grant (G2009-STAR-B1) J.E. Chambers (PI) 6/1/10-5/31/16 ($500,000)
Sponsor: EPA

Title: New Environmental Public Health Indicator Linking Organochlorine Compounds and Type 2 Diabetes

Role: Co-Investigator (M.K. Ross)

Goals: The goal of this project is to characterize novel biomarkers for the development of type 2 diabetes in humans. My role is to quantify urinary isoprostanes, a biomarker of oxidative stress, by LC-MS/MS.

COMPLETED RESEARCH SUPPORT

Grant: NIH 1R15ES015348-01A1 **M.K. Ross (PI)** 8/1/07-7/31/11 ($214,500)
Title: Effect of Organophosphate Exposure on Cholesteryl Ester Hydrolase
Role: Principal Investigator
Description: These studies will determine if bioactive metabolites (oxons) of three environmentally relevant organophosphate insecticides can interfere with cholesterol metabolism in cultured human macrophage foam cells.

Grant: NIH R15 ES015348-01A1S1 (Competitive supplement) **M.K. Ross (PI)** 9/25/09-7/31/10 ($67,200)
Title: Effect of Organophosphate Exposure on Cholesteryl Ester Hydrolase
Role: Principal Investigator
Description: It will be determined if the endocannabinoid tone of vessel wall macrophages can be significantly perturbed by chronic exposure to bioactive OP metabolites, thus resulting in an activated endocannabinoid system that modulates cholesterol metabolism in macrophages.

Grant: NIH 1R15ES015348-01A1S2 (Admin. supplement) **M.K. Ross (PI)** 9/3/09-7/31/11 ($71,500)
Title: Effect of Organophosphate Exposure on Cholesteryl Ester Hydrolase
Role: Principal Investigator
Description: This administrative supplement will extend the aims of our parent grant to study the effects of organophosphate (OP) pesticides on other genes and proteins besides CES1 that participate in cholesterol metabolism. The effects of OP pesticides on the abundance and activities of these proteins in cholesterol-loaded human THP1 macrophages using RT-PCR, west-

ern blotting, and functional assays (e.g., cholesterol efflux and cholesterol mass determination) will be examined.

Grant: NIH R21ES015107-01 J.E. Chambers (PI) 9/22/06-8/31/11 ($628,986)
Title: Relationship of Blood Esterases, Pesticide Exposure and Cardiovascular Disease
Role: Co-Principal Investigator (**M.K. Ross**)
Description: The goal of this project is to solidify an interdisciplinary team of basic and clinical researchers in the Center for Environmental Health Sciences at Mississippi State University for research into the environmental factors contributing to the higher mortality of cardiovascular disease in the Deep South and among African-Americans, and to position this team for participation in larger-scale on-going multi-institutional epidemiological studies.

Grant: R21ES015107 (Admin. supplement) J.E. Chambers (PI) 6/1/09-5/31/11 ($247,640)
Title: Relationship of Blood Esterases, Pesticide Exposure and Cardiovascular Disease
Role: Co-Principal Investigator (**M.K. Ross**)
Description: The current grant investigates several risk factors for CVD in African American and Caucasian southerners. This supplement will allow 2 additional risk factors (the presence of type 2 diabetes and of legacy organochlorine pesticides) to be investigated in the cohort's blood samples.

Grant: Basic Sciences/CVM/MSU Internal Grant (competitive) M.K. Ross (PI) 7/1/09-6/30/10 ($13,000)
Title: Knockdown of Carboxylesterases (CEs) by Chemical Inhibitors: Uncovering Endogenous Substrates for CEs
Role: Principal Investigator (**M.K. Ross**)
Description: The goal of this study is to use small-molecule inhibitors of carboxylesterases (CEs) to study their physiologic function in mice and to identify endogenous substrates of this hydrolytic enzyme.

Grant: NIH/NCRR P20RR017661 (COBRE grant, Project 5) J.E. Chambers (PI) 1/1/04-6/30/08 ($351,125)
Grant Title: Pesticide Toxicity to the Nervous and Endocrine Systems
Role: Principal Investigator of Project 5, "Biotransformation and Pharmacokinetics of Pyrethroid Insecticides". (**M.K. Ross**) This project investigated the kinetics of pyrethroid detoxication by human carboxylesterase and cytochrome P450 enzymes.
Description: This is a Center of Biomedical Research Excellence grant to promote junior faculty competitiveness and to create a competitive research center. Project 5 was one of five projects led by junior investigators.

Grant: NIH/NCRR P20RR017661 (COBRE grant, Pilot Project) J.E. Chambers (PI) 10/1/05-6/30/07 ($16,965)
Pilot Project Title: Kinetic Analyses of Site-Specific Mutants of Carboxylesterases
Role: Principal Investigator of Pilot Project.
Description: This pilot study investigated the function of specific amino acid residues located in the side-door domain of a model carboxylesterase protein (pnb CE).

Grant: NIH/NCRR P20RR017661 (COBRE grant, Pilot Project) J.E. Chambers (PI) 10/1/05-6/30/07 ($20,000)
Pilot Project Title: Effects of Prior or Concurrent Dieldrin Exposure on the Tissue Distribution and Pharmacokinetics of Atrazine in Mice: A Preliminary Study
Role: Co-Principal Investigator of Pilot Project; Nick Filipov, Principal Investigator

Description: This pilot study investigated the pharmacokinetics of the herbicide atrazine in mice. Tissue, blood, and urine levels of atrazine and its major metabolites were determined by LC-MS analysis.

Grant: USDA/CSREES M.K. Ross (PI) 6/1/06-5/31/09 ($5,000/year)
Title: Biotransformation and Pharmacokinetics of Pyrethroid Insecticides
Role: Principal Investigator
Description: This project investigated the metabolism of pyrethroids and the regulation of the detoxication enzymes in liver cells.

Grant: MSU-Research Initiation Proposal (competitive) M.K. Ross (PI)
1/1/05-12/31/05 ($10,000)
Title: Induction of Detoxification Enzymes in Liver Cells Resulting from Toxicant Exposure
Role: Principal Investigator
Description: This project investigated whether pyrethroids could induce cytochrome P450 and carboxylesterase enzymes in human liver cells.

PRESENTATIONS *(INVITED TALKS AS FACULTY MEMBER)*

Targeting the Endocannabinoid System to Enhance Immunity. Matt K. Ross. Invited talk, *Food Safety Conference,* Mississippi State University. May 12, 2015.

USING ACTIVITY-BASED PROTEIN PROBES TO INVESTIGATE SERINE HYDROLASES IN CELLS. Matt K. Ross. Presented small workshop at the *Laboratory of Food Safety* at Jiangsu Academy of Agricultural Sciences (JAAS), Nanjing, China. November, 2013.

CARBOXYLESTERASES: A MULTIFUNCTIONAL ENZYME INVOLVED IN LIPID AND PESTICIDE METABOLISM. Matt K. Ross. Invited talk at the South East Lipid Research Conference (SELRC), Callaway Gardens, Pine Mountain, GA, September 27-29, 2012.

CARBOXYLESTERASES: A MULTIFUNCTIONAL ENZYME INVOLVED IN PESTICIDE AND LIPID METABOLISM. Matt K. Ross. Invited talk at the *Institute of Food Safety* at Jiangsu Academy of Agricultural Sciences (JAAS), Nanjing, China. July, 2012.

CARBOXYLESTERASES: A MULTIFUNCTIONAL ENZYME INVOLVED IN PESTICIDE AND LIPID METABOLISM. Matt K. Ross. Invited talk at Idaho State University, College of Pharmacy. May, 2012.

CARBOXYLESTERASES: DUAL ROLES IN LIPID AND PESTICIDE METABOLISM. Matt K. Ross. Invited talk at the American Chemical Society (ACS) National Meeting, Denver, August, 2011.

HUMAN CARBOXYLESTERASES AND THEIR ROLE IN XENOBIOTIC AND ENDOBIOTIC METABOLISM. Matt K. Ross. Invited talk at the Randy Rose Memorial Symposium, Dept. of Environmental and Molecular Toxicology, North Carolina State University, March, 2007.

HUMAN CARBOXYLESTERASES AND BIOTRANSFORMATION OF PYRETHROIDS. Matt K. Ross. Invited talk at the American Chemical Society (ACS) National Meeting, Washington D.C., August, 2005.

HUMAN CARBOXYLESTERASES AND THEIR ROLE IN PYRETHROID METABOLISM. Matt K. Ross. Invited talk at the Mississippi State University COBRE Symposium, September 2005.

BIOTRANSFORMATION OF PESTICIDES BY RODENT AND HUMAN ENZYMES. Matt K. Ross. Invited seminar at the Mississippi State University Department of Biochemistry, Fall Seminar Series. November 17, 2004.

MEETING ABSTRACTS *(POSTER OR ORAL PRESENTATIONS)*

Abstracts from work since joining MSU in 2004:

M.K. Ross, L.C. Mangum, J.H. Lee, X. Hou, A. Borazjani, and J.A. Crow. *Chemical Biology and Toxicology of Human Carboxylesterase 1 in Macrophages.* Presented at the *American Chemical Society* meeting, Philadelphia, PA. August 21-25, 2016.

J.H. Lee, A. Borazjani, E. Kummari, M.J. Edelmann, and M.K. Ross. *Targeting the Endocannabinoid System to Enhance Innate Immunity Using Chemoproteomics.* Presented at the *American Society for Mass Spectrometry* meeting, San Antonio, TX. June 7-10, 2016.

E.C. Meek, J.A. Crow, L.H. Mangum, M.K. Ross, R.W. Wills, and J.E. Chambers. *Serum levels of the organochlorine (OC) compound DDE and its possible association with type 2 diabetes (T2D) in Mississippians.* Presented at the *Society of Toxicology* meeting, New Orleans, LA, March 13-17, 2016.

S. Kondakala, C. Mulligan, J.H. Lee, M.K. Ross, and G.E. Howell. *Role of the hepatic endocannabinoid system in chlorpyrifos-induced lipid accumulation in McArdle-RH7777 cells.* Presented at the *Society of Toxicology* meeting, New Orleans, LA, March 13-17, 2016.

E. Kummari, J. H. Lee, A. Borazjani, M. Edelmann, and M.K. Ross. *Characterization of Serine Hydrolases Using Chemoproteomic Profiling Approach in Chicken Macrophages with Salmonella Infection.* Presented at the *American Society of Microbiology* meeting, New Orleans, LA. May 30-June 2, 2015.

Evangel Kummari, Navatha Alugubelly, Jung Hwa Lee, Lauren Mangum, Abdolsamad Borazjani, Matthew K. Ross, and Mariola J. Edelmann. *Characterization of prostaglandins released from human macrophages infected with enteric bacteria.* Presented at the *Southeast Institute of Metabolomics*, University of Florida, Gainsville, May 13-14, 2015.

A.T. Matthews, A.Borazjani, L.C. Mangum and M.K. Ross. ENHANCED OXIDATIVE STRESS MODULATES ENDOCANNABINOID TONE. 2015 *University of Alabama, Birmingham Cardiovascular Symposium.*

A.T. Matthews, A.Borazjani, L.C. Mangum and M.K. Ross. ENHANCED OXIDATIVE STRESS MODULATES ENDOCANNABINOID TONE. 2015 *Experimental Biology* meeting, Boston, MA.

L.C. Mangum, J.A. Crow, A. Borazjani, and M.K. Ross. CHOLESTEROL HOMEOSTASIS IS REGULATED BY CARBOXYLESTERASE 1 IN MACROPHAGE FOAM CELLS. 2015 Society of Toxicology meeting, San Diego, CA.

B.F. Kaplan, B. Szafran, A. Borazjani, J.H. Lee and M.K. Ross. LPS SUPPRESSES SPLEEN SERINE HYDROLASE ACTIVITY AND 2-ARACHIDONYLGLYCEROL (2-AG) HYDROLYSIS: A POSSIBLE MECHANISM TO REGULATE INFLAMMATION. 2015 Society of Toxicology meeting, San Diego, CA.

L. Mangum, G. Howell, M.K. Ross, S. Pruett, J. Chambers, J. Stokes. P,P'-DDE ALTERS MACROPHAGE REACTIVITY *IN VITRO*AND INDUCES MONOCYTE/MACROPHAGE RECRUITMENT TO THE STROMAL VASCULAR FRACTION (SVF) OF ADIPOSE TISSUE IN C57BL/6 MALE MICE. 2015 Society of Toxicology meeting, San Diego, CA.

A.T. Matthews, A.Borazjani, R. Wang and M.K. Ross. INCREASED OXIDATIVE STRESS ENHANCES ENDOCANNABINOID TONE. 2014 *Experimental Biology* meeting, San Diego, CA.

L.C. Mangum,A. Borazjani, J.A. Crow, and M.K. Ross. BIOACTIVE LIPID METABOLISM BY CARBOXYLESTERASE 1 (CES1) IN MACROPHAGES. 2014 *Experimental Biology* meeting, San Diego, CA.

Matthews A.T., Borazjani A., Wang R., and Ross, M.K. ENHANCING 2-ARACHIDONYLGLYCEROL BIOSYNTHESIS VIA OXIDATIVE STRESS. 2013 Annual Sigma Xi Meeting, November, Research Triangle Park, NC.

Ammari M., Pharr T., Ross M.K., Pinchuk G., Pinchuk, L. MITOCHONDRIAL DYSFUNCTION ASSOCIATED WITH BOVINE VIRAL DIARRHEA VIRUS CYTOPATHOGENICITY. 2013 10th International Veterinary Immunology Symposium, Milan, Italy, Aug 28-Sept 1.

L.C. Mangum, J.E. Chambers, and M.K. Ross. ACTIVATION OF HUMAN MONOCYTIC NADPH OXIDASE BY CHLORINATED CYCLODIENE INSECTICIDES. 2013 Society of Toxicology meeting, San Antonio, TX.

Carr, R.C., Adams A.L., Kepler D.R., Ward A.B., and Ross, M.K. INDUCTION OF ENDOCANNABINOID LEVELS IN JUVENILE RAT BRAIN FOLLOWING DEVELOPMENTAL CHLORPYRIFOS EXPOSURE. 2013 Society of Toxicology meeting, San Antonio, TX.

Lin, Z., Fisher, J.W., Wang, R., Ross, M.K., Filipov, N.M. ESTIMATION OF PLACENTAL AND LACTATIONAL TRANSFER AND TISSUE DISTRIBUTION OF ATRAZINE AND ITS MAIN METABOLITES IN THE RAT DAM, FETUS, AND NEONATE WITH PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING. 2013 Society of Toxicology meeting, San Antonio, TX.

Cummings T., Bennett L., and Ross M.K. ALBENDAZOLE TISSUE DEPLETION STUDY IN CHICKENS. 2012 American Veterinary Medical Association (AVMA) national meeting, San Diego, CA.

Borazjani A., Crow J.A., Wang R., and Ross M.K. MACROPHAGES AND TOXICANTS: EFFECTS ON CHOLESTEROL EFFLUX. 2012 Society of Toxicology meeting, San Francisco, CA. *The Toxicologist* **111** (S1): Abstract # 1518.

Carr R.L., Adams A.L., Kepler D.R., Ward A.B., and Ross M.K. PATTERN OF INHIBITION OF BRAIN ENDOCANNABINOID METABOLIZING ENZYMES FOLLOWING DEVELOPMENTAL CHLORPYRIFOS EXPOSURE. 2012 Society of Toxicology meeting, San Francisco, CA. *The Toxicologist* **111** (S1): Abstract # 2565.

Carr R.L., Ward A.B., and Ross M.K. REPEATED DEVELOPMENTAL CHLORPYRIFOS EXPOSURE INCREASES ENDOCANNABINOID LEVELS IN THE BRAIN OF JUVENILE RATS. 2011 Society of Toxicology meeting, Washington, DC. *The Toxicologist* **110** (S1): Abstract # 1325.

Ross M.K., Borazjani A., and Potter P.M. INACTIVATION OF ENDOCANNABINOID METABOLISM IN HUMAN THP1 MACROPHAGES FOLLOWING EXPOSURE TO ACTIVATED ORGANOPHOSPHOTHIONATES. 2011 Society of Toxicology meeting, Washington, DC. *The Toxicologist* **110** (S1): Abstract # 2086.

Crow J.A., Bittles V., Herrring K., Borazjani A., Potter P.M., and Ross M.K. STUDY OF THE INHIBITION OF RECOMBINANT HUMAN CARBOXYLESTERASE 1 AND 2 BY CHLORPYRIFOS OXON, PARAOXON, AND METHYL PARAOXON. 2011 Society of Toxicology meeting, Washington, DC. *The Toxicologist* **110** (S1): Abstract # 2098.

Sachidananda Mishra, Deepak R. Mishra, Craig Tucker, Matthew K. Ross A QUASI-ANALYTICAL ALGORITHM TO QUANTIFY PHYCOCYANIN CONCENTRATION IN CYANOBACTERIAL ALGAL BLOOMS. 2011 Northern Gulf Institute Annual Conference.

Ross M.K., Borazjani A., Potter P.M., and Xie S. METABOLISM OF PROSTAGLANDIN GLYCERYL ESTERS BY HUMAN CARBOXYLESTERASES, CES1 AND CES2, AND ITS INHIBITION BY BIOACTIVE METABOLITES OF ORGANOPHOSPHATE INSECTICIDES. Poster abstract C122 966.10. *Experimental Biology* meeting, Anaheim, CA, April 24-28, 2010.

Carr R.L. and Ross M.K. EFFECT OF DEVELOPMENTAL CHLORPYRIFOS EXPOSURE ON ENDOCANNABINOID METABOLIZING ENZYMES IN THE BRAIN OF JUVENILE RATS. 2010 Society of Toxicology meeting, Salt Lake City, UT. *The Toxicologist* **109** (S1): Abstract # 168.

Ross M.K., K. Herring, S. Xie, P.M. Potter, and J.A. Crow. INHIBITORY EFFECTS OF OXYSTEROLS AND SATURATED AND UNSATURATED FATTY ACIDS ON HUMAN CARBOXYLESTERASE 1 AND THP1 MONOCYTE/MACROPHAGE HYDROLYTIC ACTIVITYES. 2009 Society of Toxicology meeting, Baltimore, MD. *The Toxicologist* **108** (S1): Abstract # 905.

Ross M.K., A. Borazjani, S. Xie, and P.M. Potter. FROM XENOBIOTICS TO ENDOBIOTICS: EFFICIENT HYDROLYSIS OF THE ENDOCANNABINOID 2-ARACHIDONOYLGLYCEROL BY HUMAN CARBOXYLESTERASES 1 AND 2. 2008 Society of Toxicology meeting, Seattle, WA. *The Toxicologist* **102** (S1): Abstract # 301.

Crow J.A., K. Hardin, A. Borazjani, and M.K. Ross. EFFECT OF THE LIPID PEROXIDIATION PRODUCT 4-HYDROXY-2-NONENAL ON ESTERASE AND LIPASE ACTIVITIES IN HUMAN THP-1 MONOCYTES/MACROPHAGES. 2008 Society of Toxicology meeting, Seattle, WA. *The Toxicologist* **102** (S1): Abstract # 2053.

Davis M.K., M. Russak, M.K. Ross, and J.E. Chambers. ASSESSING POTENTIAL EXPOSURE TO TRANSFERABLE INSECTICIDE RESIDUES FROM THE FUR OF DOGS TREATED WITH A SPOT-ON FLEA CONTROL PRODUCT CONTAINING THE PYRETHROID INSECTIDIE PERMETHRIN. 2008 Society of Toxicology meeting, Seattle, WA. *The Toxicologist* **102** (S1): Abstract # 1481.

Filipov N.M., M.K. Ross, L.M. Pinchuk, A. Borazjani and A. Coban. METABOLISM AND HEALTH EFFECTS OF ATRAZINE EXPOSURE IN THE MOUSE. 2008 Society of Toxicology meeting, Seattle, WA. *The Toxicologist* **102** (S1): Abstract # 1985.

Godin S.J., M.F. Hughes, M.K. Ross and M.J. DeVito. METABOLISM OF PYRETHROID PESTICIDES BY RAT AND HUMAN CYP450S AND SERUM. 2007 Society of Toxicology meeting, Charlotte, NC. *The Toxicologist* **96** (S1): Abstract # 1980.

Streit T.M., A. Borazjani, S.E. Lentz and M.K. Ross. EXAMINATION OF THE PROPOSED "SIDE DOOR" IN THE XENOBIOTIC METABOLIZING ENZYME CARBOXYLESTEARASE. 2007 Society of Toxicology meeting, Charlotte, NC. *The Toxicologist* **96** (S1): Abstract # 349.

Ross M.K., A. Borazjani, J.A. Crow, and M.P. Patricelli. EVALUATION OF THE CARBOXY-LESTERASE PHENOTYPE IN HUMAN LIVER. 2007 Society of Toxicology meeting, Charlotte, NC. *The Toxicologist* **96** (S1): Abstract # 350.

Filipov N. M., T.L. Jones, and M.K. Ross. PHARMACOKINETICS AND TISSUE DISTRIBU-TION OF ATRAZINE IN MALE C57BL/6 MICE. 2007 Society of Toxicology meeting, Char-lotte, NC. *The Toxicologist* **96** (S1): Abstract # 2034.

Crow J.A., B.L. Middleton, and M.K. Ross. INHIBITION OF CHOLESTERYL ESTER HYDRO-LASE IN THP-1 CELLS BY ORGANOPHOSPHORUS OXONS. 2007 Society of Toxicology meeting, Charlotte, NC. *The Toxicologist* **96** (S1): Abstract # 2121.

Streit T.M., A. Borazjani, S.E. Lentz and M.K. Ross. EXAMINATION OF THE "SIDE DOOR" IN THE XENOBIOTIC METABOLIZING ENZYME CARBOXYLESTEARASE. 2006 SouthCen-tral Regional meeting of the Society of Toxicology, Monroe, LA.

Ross M.K., A. Borazjani, P.M. Potter, and T. Streit. METABOLISM OF PYRETHROIDS BY HUMAN CARBOXYLESTERASES. 2006 ISSX meeting, Puerto Rico.

Ross M.K., A. Borazjani, P.M. Potter, and T. Streit METABOLISM OF PYRETHROIDS BY HUMAN CARBOXYLESTERASES. 2006 COBRE/INBRE symposium, Washington, DC. This was a "highlighted poster" at the meeting.

Ross M.K., S.E. Lentz, and A. Borazjani. CHARACTERIZATION OF TWO RAT CAR-BOXYLESTERASES INVOLVED IN PYRETHROID METABOLISM. 2006 Society of Toxicology meeting, San Diego, CA. *The Toxicologist* **90** (S1): Abstract # 694.

Davis M.K., M. Russak, J.W. Tyler, J.S. Boone, M.K. Ross, and J.E. Chambers. ASSESSING EXPOSURE LEVELS OF CHILDREN TO FLEA CONTROL INSECTICIDES (CHLORPYRIFOS, TETRACHLORVINPHOS, AND PERMETHRIN) FROM THE FUR OF DOGS. 2006 Society of Toxicology meeting, San Diego, CA. *The Toxicologist* **90** (S1): Abstract # 862.

Godin S.J., M.F. Hughes, M.J. DeVito, and M.K. Ross. SPECIES DIFFERENCES IN THE METABOLISM OF PYRETHROID PESTICIDES IN RAT AND HUMAN LIVER MICROSOMES. 2006 Society of Toxicology meeting, San Diego, CA. *The Toxicologist* **90** (S1): Abstract # 1202.

Dail M., S. Burgess, M.K. Ross, and J. Chambers. EFFECTS OF DIELDRIN AND PHENOBARBITAL ON THE LEVELS OF MESSENGER RNA OF TOXICOLOGICALLY IMPORTANT GENES. 2006 Society of Toxicology meeting, San Diego, CA. *The Toxicologist* **90** (S1): Abstract # 1825.

Ross M.K., S.E. Lentz, and A. Borazjani. CHARACTERIZATION OF TWO RAT CARBOXYLESTERASES INVOLVED IN PYRETHROID METABOLISM. 2005 South Central Chapter Regional meeting of the Society of Toxicology, Little Rock, AR.

Ross M.K., P.M. Potter, and A. Borazjani. HYDROLYTIC METABOLISM OF PYRETHROIDS BY HUMAN CARBOXYLESTERASES AND RODENT AND HUMAN LIVER MICROSOMES. 2005 Society of Toxicology meeting, New Orleans, LA. *The Toxicologist* **84** (S1): Abstract # 1569.

Ross. M.K., Potter, P.M., and Borazjani, A. HYDROLYTIC METABOLISM OF PYRETHROIDS BY HUMAN CARBOXYLESTERASES AND RODENT AND HUMAN LIVER MICROSOMES. 2004 South Central Chapter Regional meeting of Society of Toxicology, Mississippi State University.

Abstracts from postdoctoral and graduate research work:

Ross M.K., R. Tornero-Velez, C. Granville, A. Gold, K. Funasaka, M.V. Evans, and D.M. DeMarini. METABOLISM AND BIOACTIVATION OF 1,1- AND 1,3-DICHLOROPROPENE. 2004 International Society for the Study of Xenobiotics (ISSX) meeting, Vancouver, BC.

Ross M.K., C.R. Eklund, and R.A. Pegram. COMPARISON OF DETOXIFICATION AND BIOACTIVATION PATHWAYS FOR BROMODICHLOROMETHANE IN THE RAT. 2004 Society of Toxicology meeting, Baltimore, MD. *The Toxicologist*: Abstract # 1452.

Pegram, R.A., M.K. Ross, T.L. Leavens, J.W. Allis, B.C. Blount, and G. Zhao. BROMODICHLOROMETHANE TOXICOKINETICS: LINKING EXPOSURE TO EFFECT. Presented at the 2002 U.S.EPA Science Fair, May 1-2, Washington, D.C.

Ross M.K. and R.A. Pegram. COMPARISON OF RATES OF GLUTATHIONE (GSH)-CONJUGATION OF TRIHALOMETHANES. 2002 Society of Toxicology meeting, Nashville, TN. *The Toxicologist, Abstract # 1118.*

Ross M.K. and R.A. Pegram. GLUTATHIONE (GSH)-DEPENDENT METABOLISM OF THE DISINFECTION-BY-PRODUCT BROMODICHLOROMETHANE (BDCM). 2001 International Society for the Study of Xenobiotics (ISSX) meeting, Munich, Germany. *Drug Metab. Rev.,* **33** (Suppl. 1) 342.

Ross M.K. and R.A. Pegram. GLUTATHIONE *S*-TRANSFERASE-MEDIATED METABOLISM OF BROMODICHLOROMETHANE. 2001 Society of Toxicology meeting, San Francisco, CA. *The Toxicologist, Abstract # 438.*

Pegram, R.A and M.K. Ross. DNA BINDING POTENTIAL OF BROMODICHLOROMETHANE MEDIATED BY GLUTATHIONE S-TRANSFERASE THETA 1-1. 2001 Society of Toxicology meeting, San Francisco, CA. *The Toxicologist, Abstract # 439.*

Ross. M. K., B. Said, and R.C. Shank. NON-ADDITIVE DNA-DAMAGING EFFECTS OF GEN-

OTOXINS IN MIXTURE: 2. COVALENT BINDING TO DNA. 1999 Society of Toxicology meeting, New Orleans, LA. *The Toxicologist, Abstract # 1090.*

Ross M.K. and R.C. Shank. MODULATION OF ADDUCT FORMATION AFTER EXPOSURE OF OLIGONUCLEOTIDES CONTAINING PRE-EXISTING SITE-SPECIFIC ADDUCTS TO BULKY CARCINOGENS (1996) Presented at the Histopathobiology of Neoplasia Workshop, sponsored by the American Association of Cancer Research, Keystone, CO.

Shank R.C., M.K. Ross, B. Said, and T. Salib,T. MODULATION OF DNA ADDUCT FORMATION AFTER EXPOSURE OF DNA TO SMALL AND BULKY CARCINOGENS. 1995 International Society of Toxicology meeting, Seattle, WA. *The International Toxicologist, Abstract # 12-PD-10.*

Menzel D.B., M.K. Ross, S.V. Oddo, and H. Roth. A PRELIMINARY PB-PK MODEL OF INGESTED ARSENATE IN SWISS-WEBSTER MICE. 1994 Society of Toxicology meeting, Dallas, TX. *The Toxicologist, Abstract # 68.*

Ross M.K., D. Meacher, S.V. Oddo, R.E. Rassmussen, and D.B. Menzel. COMPARATIVE STUDIES OF FERRET AND RAT GLUTATHIONE *S*-TRANSFERASE SUBUNITS. 1994 Society of Toxicology meeting, Dallas, TX. *The Toxicologist, Abstract # 1326.*

PROFESSIONAL DEVELOPMENT SINCE 2004 (CONTINUING ED. COURSES/TRAINING):

Course title: *Reactive Oxygen Species.* March 2009. SOT meeting, Baltimore, MD.

Course title: *Metabolomics.* November 2008. Applications of Mass Spectrometry to the Clinical Laboratory meeting, San Diego, CA.

Course title: *Human Polymorphic Responses to Drugs.* October 2006. ISSX meeting, Puerto Rico.

Course title: *Xenobiotic Transporters.* March 2006. SOT meeting, San Diego, CA.

Course title: *Fundamentals of Nanotechnology: Chemistry, Exposure, and Health Effects.* March 2005. SOT meeting, New Orleans, LA.

Course title: *Regulation of Cytochrome P450 and Transporters.* August 2004. ISSX meeting, Vancouver, BC.

Course title: *Computational Biology, Dose and Response*, March 2004. SOT meeting, Baltimore, MD.

Four days of training on LC-MS instrument at the Thermo Finnigan Training Institute, W. Palm Beach, FL. July 26-29, 2004.

ACTIVE OUTSIDE COLLABORATORS:

Philip M. Potter, Ph.D.
Department of Molecular Pharmacology
St. Jude Children's Research Hospital
Memphis, TN

Nikolay (Nick) M. Filipov, Ph.D.
Department of Pharmacology and Physiology
College of Veterinary Medicine
University of Georgia
Athens, GA

Ran Wang, Ph.D.
Institute of Food Safety
Jiangsu Academy of Agricultural Sciences (JAAS)
Nanjing, China

TEACHING (FTE 15%)

GRADUATE COURSES

Course: Mechanisms of Toxic Action/Molecular Toxicology (CVM 8543, 3 h)
Instructor of record: Dr. Matt K. Ross
Semesters: Fall, 2009; Fall, 2011; Fall 2015, 2016 (problems-based course); Fall 2016
Role: Taught the majority of lectures in this course (85% of the lectures)

Course: Organ Systems Toxicity II (CVM 8533, 3 h)
Instructor of record: Dr. Russell Carr
Semesters: Spring, 2009; Spring, 2011
Role: Taught sections on endocrinology/diabetes/cardiovascular (16% of the lectures; new lectures prepared on metabolic syndrome diseases and atherosclerosis)

Course: Organ Systems Toxicity I (CVM 8523, 3 h)
Instructor of record: Dr. Russell Carr
Semesters: Spring, 2006; Spring, 2008; Spring, 2010; Spring, 2012
Role: Taught sections on liver physiology/pathophysiology (16% of the lectures)

Course: Mechanisms of Toxic Action (CVM 8543, 3 h)
Instructor of record: Dr. Russell Carr
Semesters: Spring, 2005; Spring, 2007
Role: Taught sections on xenobiotic metabolism/mutagenesis/carcinogenesis (40% of the lectures; new lectures prepared for the section on biotransformation, genotoxicity, mutagenesis, and carcinogenesis)

Course: Current Literature in Toxicology (Special topics course, 1 h)
Instructor of record: Dr. Matt K. Ross
Semesters: Fall, 2005
Role: Coordinated a journal club for graduate students; presented two journal clubs to the students during the course

Course: Graduate Student Seminar (CVM 8011, 1 h)
Instructor of record: Dr. Matt K. Ross
Semesters: Fall, 2004–Spring, 2007 (6 semesters)
Role: Coordinated the CVM graduate student seminar series

GUEST LECTURES IN CVM GRADUATE COURSES

Two lectures on pharmacokinetics in Dr. Cory Langston's graduate *Pharmacology* course, CVM 8403 (Spring, 2004; Spring, 2007)

Four lectures on signal transduction pathways in Drs. Pharr's and Pinchuk's *Advanced Immunology* graduate course, CVM 8303 (Spring, 2009; Spring, 2011; Spring, 2012; Spring, 2013; Spring 2014)

DIRECTED INDIVIDUAL STUDY

Course: Techniques in Analytical Toxicology
Instructors of record: Dr. Matt K. Ross/Dr. Cory Langston
Semester: Spring, 2005
Student: Jay Pittman, 2 hour course

STUDENT AND POSTDOCTORAL ADVISEMENT

Master's students (Major Professor):

Tim Streit, tenure in lab 8/05-8/07
Graduated: August, 2007
Current position: Assistant Study Director, Covance Pharmaceuticals, Madison, WI

Shuqi Xie, tenure in lab 8/07-12/10
Graduated: December, 2009
Current position: Research Associate, Department of Hygiene Toxicology, Preventive Medical College, Third Military Medical University, Chongqing, China.

Ph.D. students (Major Professor):

Lee Magnum, tenure 8/09-present
Anberitha Matthews, tenure 8/11-present (Awarded NIH pre-doctoral fellowship, August 2014, F31 HL122082-01A1)

Postdoctoral Fellows:

Dr. Kristen Funk (tenure: 1/11-7/11; current position, Assistant Professor, James Madison University, VA)
Dr. Ran Wang (tenure: 8/11-8/13; current position, Professor, JAAS, Nanjing, China)
Dr. Jung Hwa Lee (tenure: 9/13-present)
Dr. Xiang Hou (tenure: 1/16-present)

Undergraduate students:

Katye Herring, tenure in lab 8/07-12/09
Awarded a *Shackouls Undergraduate Student Research Award* (summer '08)
Currently: Medical student, University of Mississippi, Jackson, MS

Victoria Bittles, tenure in lab 8/09-present
Currently: Senior at Mississippi State University (still works in my lab)

Jayne Carlson, tenure in lab 1/10-5/10
Currently: Works for a health-care non-profit organization in Mississippi

Claire Dagre, tenure in lab 9/09-5/10.
Currently: *Human Vaccine Institute*, Duke University, Durham, NC

Antonio Ward, tenure in lab 5/10-8/10.
Currently: Toxicology graduate student, Mississippi State University

Ms. Herring, Bittles, Carson, and Dagre and Mr. Ward were supported by my R15 grant

Veterinary students – performed summer research in the lab:

Shellaine Lentz, tenure in lab 5/05-8/05; also 1/07-5/07
Lloyd Reitz, tenure in lab 5/06-8/06
Kate Lightner, tenure in lab 5/07-8/07

Kim Pluta, tenure in lab 5/09-8/09
[Stipend support for the veterinary students was provided by NIH T35RR007071 (Ainsworth, Lawrence, PIs)]

Graduate student committees (MS or PhD):

Past students:
J.E. Moran, MS (advisor: J.E. Chambers)
Frank Johnson, PhD (advisor: R.L. Carr)
Jay Pittman, PhD (advisor: J.E. Chambers)
Tim Streit, MS (advisor: M.K. Ross)
Shuqi Xie, MS student (advisor: M.K. Ross)
Paul Eden, PhD student (advisor: J.E. Chambers)
Chelsea Macintosh, MS student (advisor: J. Warnock)
Guohua Yang, MS student (advisor: H. Wan)
Ron Pringle, PhD student (advisor: J.E. Chambers)

Current students: Antonio Ward, PhD student (advisor: J.E. Chambers)

SERVICE (FTE 15%)

EXTERNAL REVIEW PANELS:

Invited member, USEPA Federal Insecticide, Fungicide and Rodenticide Act Scientific Advisory Panel Meeting (August 16-17, 2007) on "Assessing Approaches for the Development of PBPK Models of Pyrethroid Pesticides" held at the Environmental Protection Agency Conference Center, Arlington, VA.

Invited member, NIOSH Study Section, Philadelphia, PA, June 6-10, 2011.

Invited member, Agricultural Health Study (AHS) National Advisory Panel, Rockville, MD, March 1-2, 2012.

Invited member, NIH Study Section, Special Emphasis Panel (review of R15 grants), November 29, 2012.

Invited member, NIH Study Section, Systemic Injury by Environmental Exposures, February 5-6, 2013.

Invited member, NIH Study Section, Systemic Injury by Environmental Exposures, November 11-12, 2013.

International Agency for Research on Cancer (IARC) Monograph vol. 112 Writing Team (March, 2015)

International Agency for Research on Cancer (IARC) Monograph vol. 117 Writing Team (October, 2016) – *subgroup chair*, Mechanisms subgroup.

Invited grant reviewer, Austrian Science Fund (November 2015, April 2016)

REVIEWER/EDITORIAL BOARD FOR JOURNALS:

Ad-hoc reviewer for scientific journals (number of manuscripts reviewed for each journal is indicated in parentheses; updated September 2013):

ACS Books (1), ACS Chemical Neuroscience (1), Analytical Biochemistry (1), Biochemical Pharmacology (4), BMC Genomics (1), BMC Research Notes (2), Cardiovascular Toxicology (1), Chemico-Biological Interactions (16), Chemical Research in Toxicology (3), Chemistry &

Biology (1), Comparative Biochemistry and Physiology (1), Current Drug Metabolism (1), Environmental and Molecular Mutagenesis (1), Food and Chemical Toxicology (2), Food and Function (1), Journal of Agricultural and Food Chemistry (2), Journal of Biochemical and Molecular Toxicology (2), Journal of Child and Adolescent Psychopharmacology (1), Insect Biochemistry and Molecular Biology (1), International Journal of Toxicology (1), Life Sciences (1), Molecules (1), Nature Chemical Biology (1), Plos One (2), Toxicology and Applied Pharmacology (3), Toxicology In Vitro (3), Toxicological Sciences (5), Toxicology (1), Pesticide Biochemistry and Physiology (1), Journal of Bacteriology (1), African Journal of Biotechnology (1), Ecotoxicology and Environmental Safety (2), Journal of Pharmacology and Experimental Therapeutics (1).

Editorial board member (invited), *Toxics* (2013-present)

UNIVERSITY SERVICE:

-- Hazardous Waste Committee (Member, Fall 2005 – Fall 2006)

-- Life Sciences and Biotechnology Institute (LSBI) Task Force (Member, Spring 2007)

-- Radiation, Chemical and Laboratory Safety Committee (Member, Fall 2006 – current)

-- Chair, Radiation, Chemical and Laboratory Safety Committee (Fall 2013 – current)

-- Search committee, Environmental Health and Safety Director position (Member, Spring 2013)

DEPARTMENT/COLLEGE SERVICE:

-- *Research Advisory Committee*, College of Veterinary Medicine, MSU (2010-present)

-- *College Tenure and Promotion Committee*, College of Veterinary Medicine, MSU (2011-present)

-- *Lipidomics Research Program* Director, College of Veterinary Medicine, MSU (2011-present)

-- Ad-hoc selection committee to review applications of veterinary students applying for positions as NIH-funded summer researchers at the CVM (Spring 2004)

-- Interviewer of veterinary student applicants (Spring 2006)

-- Faculty Search Committees (Toxicology positions), Department of Basic Sciences (Spring 2008, Fall 2012, Spring 2013); (Chair of search committees; Fall 2012, Spring 2013)

-- Served as judge for veterinary and graduate student research presentations during CVM Research Day (Fall 2007; Fall 2008; Fall, 2011; Fall 2012).

-- Advisor and consultant for investigators, students, and staff members in the Center for Environmental Health Sciences regarding bioanalytical needs, experimental design, and instrumentation. Advice was given on the use of specific analytical platforms, including GC-MS, LC-MS, and LC-UV. Played a significant role in determining which instrumentation should be purchased by the Center for bioanalytical needs.

-- In-house reviewer of manuscripts at the CVM (average of 3 per year).

-- Research Strategic Planning committee, College of Veterinary Medicine, Mississippi State University (2010).

CLINICAL / DIAGNOSTIC SERVICE:

Performed LC-MS analyses of dog and bird blood for the presence of specific antibiotics as part of a clinical study (PI; Dr. Cory Langston, College of Veterinary Medicine, MSU). 2005-2006.

Performed LC-MS/MS analyses of dog blood for dantrolene and its major metabolite as part of a clinical study (PI; Drs. Todd Archer/Andrew Mackin, College of Veterinary Medicine, MSU). 2011-1012.

Performed LC-MS/MS analyses of horse blood for nadolol as part of a clinical study (PI; Dr. Chipper Swiderski, College of Veterinary Medicine, MSU). 2011-2012.

Performed LC-MS analyses of bovine liver samples for the presence of atrazine residues (PI; Dr. John Roberts, College of Veterinary Medicine, Auburn University). 2008.

OTHER:

Judge for student poster competition, fall meeting of the South Central Chapter of the Society of Toxicology Meeting held at Mississippi State University (October, 2004).

Tips to get your Science Published in Peer-reviewed English Language Journals. Matt K. Ross, 7 lectures given at the Jiangsu Academy of Agricultural Sciences (JAAS), Nanjing, China. June, 2015.

REFERENCES:

1. Phil M. Potter, PhD, Member, Department of Chemical Biology and Therapeutics, St. Jude Children's Research Hospital, Memphis, TN. Email: phil.potter@stjude.org. Tel. (901) 595-2825

2. Nikolay (Nick) M. Filipov, PhD, Associate Professor, Department of Pharmacology and Physiology, College of Veterinary Medicine, University of Georgia. Email: filipov@uga.edu. Tel. (706) 542-3014

3. Michael Devito, PhD, Head, Experimental Toxicology Group, National Toxicology Program, National Institutes of Environmental Health, Research Triangle Park, NC. Email: devi-tom@niehs.nih.gov. Tel. (919) 541-4142

003031

From: Kathryn M. Forgie
To: Ross, Matthew
Subject: Fwd: Cancer induced by Glyphosate
Date: Monday, June 8, 2015 5:33:40 PM

>
> Dear Dr. Ross: I read, with great interest, the recent IARC classification of glyphosate, and see that you were involved in studying this issue. I also have read, or more accurately, attempted to read, some of your work on organochlorines leading to disease state through the mechanism of systemic oxidative stress. I am a lawyer representing persons who have developed cancer after such exposure and am hoping I can arrange a time to speak with you to discuss the research and issues involved. I could meet you at a place convenient to you in Mississippi, or we could set up a time to talk on the phone - whichever is easiest for you. I look forward to hearing from you. Regards. Kathryn [redacted]
>
> Sent from my iPad

000297

DECLARATION OF INTERESTS FOR IARC/WHO EXPERTS

IARC/WHO's work on global health issues requires the assistance of external experts who **may have interests related to their expertise.** To ensure the highest integrity and public confidence in its activities, IARC/WHO requires that experts serving in an advisory role disclose any circumstances that could give rise to a potential conflict of interest related to the subject of the activity in which they will be involved.

All experts serving in an advisory role must disclose any circumstances that could represent a **potential conflict of interest** (i.e. any interest that may affect, or may reasonably be perceived to affect, the expert's objectivity and independence). You must disclose on this Declaration of Interest (DOI) form any financial, professional or other interest relevant to the subject of the work or meeting in which you have been asked to participate in or contribute towards and any interest that could be affected by the outcome of the meeting or work. You must also declare relevant interests of your immediate family members (see definition below) and, if you are aware of it, relevant interests of other parties with whom you have substantial common interests and which may be perceived as unduly influencing your judgement (e.g. employer, close professional associates, administrative unit or department).

Please complete this form and submit it to IARC/WHO Secretariat if possible at least 4 weeks but no later than 2 weeks before the meeting or work. You must also promptly inform the Secretariat if there is any change in this information prior to, or during the course of, the meeting or work. All experts must complete this form before participation in a IARC/WHO activity can be confirmed.

Answering "Yes" to a question on this form does not automatically disqualify you or limit your participation in a IARC/WHO activity. Your answers will be reviewed by the Secretariat to determine whether you have a conflict of interest relevant to the subject at hand. One of the outcomes listed in the next paragraph can occur depending on the circumstances (e.g. nature and magnitude of the interest, timeframe and duration of the interest).

The Secretariat may conclude that no potential conflict exists or that the interest is irrelevant or insignificant. If, however, a declared interest is determined to be potentially or clearly significant, one or more of the following three measures for managing the conflict of interest may be applied. The Secretariat (i) allows full participation, with public disclosure of your interest; (ii) mandates partial exclusion (i.e. you will be excluded from that portion of the meeting or work related to the declared interest and from the corresponding decision making process); or (iii) mandates total exclusion (i.e. you will not be able to participate in any part of the meeting or work).

All potentially significant interests will be **disclosed** to the other participants at the start of the activity and you will be asked if there have been any changes. A summary of all declarations and actions taken to manage any declared interests will be **published** in resulting reports and work products. Furthermore, if the objectivity of the work or meeting in which you are involved is subsequently questioned, the contents of your DOI form may be made available by the Secretariat to persons outside IARC/WHO if the Director/Director-General considers such disclosure to be in the best interest of the Organization, after consulting with you. Completing this DOI form means that you agree to these conditions.

If you are unable or unwilling to disclose the details of an interest that may pose a real or perceived conflict, you must disclose that a conflict of interest may exist and the Secretariat may decide that you be totally recused from the meeting or work concerned, after consulting with you.

Name: Matthew K. Ross
Institution: Mississippi State University
Email: [redacted]

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans
Volume 112: Some Organophosphate Insecticides
Lyon, France: 3–10 March 2015

Please answer each of the questions below. If the answer to any of the questions is "yes", briefly describe the circumstances on the last page of the form.

The term "you" refers to yourself and your immediate family members (i.e. spouse (or partner with whom you have a similar close personal relationship) and your children). "Commercial entity" includes any commercial business, an industry association, research institution or other enterprise whose funding is significantly derived from commercial sources with an interest related to the subject of the meeting or work. "Organization" includes a governmental, international or non-profit organization. "Meeting" includes a series or cycle of meetings.

000298

EMPLOYMENT AND CONSULTING

Within the past 4 years, have you received remuneration from a commercial entity or other organization with an interest related to the subject of the meeting or work?

1a Employment — Yes ☐ No ☑

1b Consulting, including service as a technical or other advisor — Yes ☑ No ☐

RESEARCH SUPPORT

Within the past 4 years, have you or has your research unit received support from a commercial entity or other organization with an interest related to the subject of the meeting or work?

2a Research support, including grants, collaborations, sponsorships, and other funding — Yes ☐ No ☑

2b Non-monetary support valued at more than US $1000 overall (include equipment, facilities, research assistants, paid travel to meetings, etc.) — Yes ☑ No ☐

2c Support (including honoraria) for being on a speakers bureau, providing speeches or training for a commercial entity or other organization with an interest related to the subject of the meeting or work? — Yes ☐ No ☑

INVESTMENT INTERESTS

Do you have current investments (valued at more than US $1000) in a commercial entity with an interest related to the subject of the meeting or work?
Please also include indirect investments such as a trust or holding company. You may exclude mutual funds, pension funds or similar investments that are broadly diversified and on which you exercise no control.

3a Stocks, bonds, stock options, other securities (e.g. short sales) — Yes ☐ No ☑

3b Commercial business interests (e.g. proprietorships, partnerships, joint ventures, board memberships, controlling interest in a company) — Yes ☐ No ☑

INTELLECTUAL PROPERTY

Do you have any intellectual property rights that might be enhanced or diminished by the outcome of the meeting or work?

4a Patents, trademarks, or copyrights (including pending applications) — Yes ☐ No ☑

4b Proprietary know-how in a substance, technology or process — Yes ☐ No ☑

PUBLIC STATEMENTS AND POSITIONS (during the past 3 years)

5a As part of a regulatory, legislative or judicial process, have you provided an expert opinion or testimony, related to the subject of the meeting or work, for a commercial entity or other organization? — Yes ☐ No ☑

5b Have you held an office or other position, paid or unpaid, where you represented interests or defended a position related to the subject of the meeting or work? — Yes ☐ No ☑

ADDITIONAL INFORMATION

6a If not already disclosed above, have you worked for the competitor of a product that is the subject of the meeting or work, or will your participation in the meeting or work enable you to obtain access to a competitor's confidential proprietary information, or create for you a personal, professional, financial or business competitive advantage? — Yes ☐ No ☑

6b To your knowledge, would the outcome of the meeting or work benefit or adversely affect interests of others with whom you have substantial common personal, professional, financial or business interests (such as your adult children or siblings, close professional colleagues, administrative unit or department)? — Yes ☐ No ☑

6c Excluding IARC/WHO, has any person or entity paid or contributed towards your travel costs in connection with this IARC/WHO meeting or work? — Yes ☐ No ☑

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6d Have you received any payments (other than for travel costs) or honoraria for speaking publicly on the subject of this IARC/WHO meeting or work? Yes ☐ No ☑

6e Is there any other aspect of your background or present circumstances not addressed above that might be perceived as affecting your objectivity or independence? Yes ☐ No ☑

7 **TOBACCO OR TOBACCO PRODUCTS** *(answer without regard to relevance to the subject of the meeting or work)*
Within the past 4 years, have you had employment or received research support or other funding from, or had any other professional relationship with, an entity directly involved in the production, manufacture, distribution or sale of tobacco or tobacco products or representing the interests of any such entity? Yes ☐ No ☑

EXPLANATION OF "YES" RESPONSES: If the answer to any of the above questions is **"yes", check above and briefly describe the circumstances on this page. If you do not describe the nature of an interest or if you do not provide the amount or value involved where relevant, the conflict will be assumed to be significant.**

Nos. 1-4, 7: Type of interest, question number and category (e.g. Intellectual Property 4.a copyrights) and basic descriptive details	**Name of company, organization, or institution**	**Belongs to you, a family member, employer, research unit or other?**	**Amount of income or value of interest (if not disclosed, is assumed to be significant)**	**Current interest (or year ceased)**
Employment/Consulting Ques. 1b	Serve on advisory panel of the Agricultural Heath Study (NCI, NIH) 2012-present	No	Travel/perdiem Honorarium ~~$2,000.00~~ $1,000.00	2012-present
Research Support Ques. 2b	Paid travel to visit Institute of Food Safety Jiangsu Academy of Agric. Sciences Nanjing, China	No	Plane fare/ lodging/food only. Honorarium $2,000.00	2013

Nos. 5-6: Describe the subject, specific circumstances, parties involved, time frame and other relevant details.

Ag. Health Study advisory panel -- provide expertise on study design/data interpretation/advice.

Travel to JAAS, Nanjing, China -- Collaboration between scientists @ JAAS and MSU (Miss State University)

CONSENT TO DISCLOSURE. By completing and signing this form, you consent to the disclosure of any relevant conflicts to other meeting participants and in the resulting report or work product.

DECLARATION. I hereby declare on my honour that the disclosed information is true and complete to the best of my knowledge.

Should there be any change to the above information, I will promptly notify the responsible staff of IARC/WHO and complete a new declaration of interests form that describes the changes. This includes any change that occurs before or during the meeting or work itself and through the period up to the publication of the final results or completion of the activity concerned.

Date: 8/7/14 Signature: [signature]

Date: _____ Signature: _____
(to be signed again at the meeting)

World Health Organization

Subgroup 4 Working Group Members

Ivan I. Rusyn (Subgroup Chair)
Veterinary Integrative Biosciences
College of Veterinary Medicine & Biomedical Sciences
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U.S. Environmental Protection Agency
3153 Rapid Falls Road
Cary, NC 27519
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[redacted]

Matthew K. Ross
College of Veterinary Medicine
Mississippi State University
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Mississippi State, MS 39762-6100
USA
[redacted]

Lauren Zeise
California Environmental Protection Agency
Reproductive and Cancer Hazard Assessment
1515 Clay Street, 16th Floor
Oakland, CA 94612
USA

[redacted]

Invited specialist

Christopher J. Portier [retired]
[redacted]

Vol 112 – Overview of assignments

Section Title	Home Section	Author
M1:1.1 Chemical and physical data	M1 Malathion	Peter P. Egeghy
M1:1.2 Production and use	M1 Malathion	Peter P. Egeghy
M1:1.3 Measurement and analysis	M1 Malathion	Peter P. Egeghy
M1:1.4 Occurrence and exposure	M1 Malathion	Lin Fritschi
M1:1.5 Regulations and guidelines	M1 Malathion	Hans Kromhout
M1:2 Studies of cancer in humans	M1 Malathion	Isabelle Baldi
M1:3 Studies of cancer in experimental animals	M1 Malathion	Gloria D. Jahnke
M1:4.1 Toxicokinetic data	M1 Malathion	Matt Ross
M1:4.2.1 Genetic and related effects	M1 Malathion	Frank LeCurieux
M1:4.2.2 Receptor-mediated effects	M1 Malathion	Lauren Zeise
M1:4.2.3 Oxidative stress, inflammation and immunosuppression	M1 Malathion	Ivan Rusyn
M1:4.2.4 Altered cell proliferation	M1 Malathion	Lauren Zeise
M1:4.2.5 Other mechanisms	M1 Malathion	Lauren Zeise
M1:4.3 Data relevant to comparisons across agents and endpoints	M1 Malathion	Matt Martin
M1:4.4 Cancer susceptibility data	M1 Malathion	Ivan Rusyn
M1:4.5 Other adverse effects	M1 Malathion	Matt Martin
M1:4.6 Mechanistic considerations	M1 Malathion	Matt Martin
M2:1.1 Chemical and physical data	M2 Parathion	Peter P. Egeghy
M2:1.2 Production and use	M2 Parathion	Peter P. Egeghy
M2:1.3 Measurement and analysis	M2 Parathion	Peter P. Egeghy
M2:1.4 Occurrence and exposure	M2 Parathion	Lin Fritschi
M2:1.5 Regulations and guidelines	M2 Parathion	Hans Kromhout
M2:2 Studies of cancer in humans	M2 Parathion	John McLaughlin
M2:3 Studies of cancer in experimental animals	M2 Parathion	Maria Consolato Sergi
M2:4.1 Toxicokinetic data	M2 Parathion	Matt Ross
M2:4.2.1 Genetic and related effects	M2 Parathion	Frank LeCurieux
M2:4.2.2 Receptor-mediated effects	M2 Parathion	Lauren Zeise
M2:4.2.3 Oxidative stress, inflammation and immunosuppression	M2 Parathion	Ivan Rusyn
M2:4.2.4 Altered cell proliferation	M2 Parathion	Lauren Zeise
M2:4.2.5 Other mechanisms	M2 Parathion	Lauren Zeise
M2:4.3 Data relevant to comparisons across agents and endpoints	M2 Parathion	Ivan Rusyn
M2:4.4 Cancer susceptibility data	M2 Parathion	Ivan Rusyn
M2:4.5 Other adverse effects	M2 Parathion	Matt Martin
M2:4.6 Mechanistic considerations	M2 Parathion	Matt Ross
M3:1.1 Chemical and physical data	M3 Diazinon	Peter P. Egeghy
M3:1.2 Production and use	M3 Diazinon	Peter P. Egeghy
M3:1.3 Measurement and analysis	M3 Diazinon	Peter P. Egeghy
M3:1.4 Occurrence and exposure	M3 Diazinon	Teresa Rodriguez
M3:1.5 Regulations and guidelines	M3 Diazinon	Hans Kromhout
M3:2 Studies of cancer in humans	M3 Diazinon	Andrea 't Mannetje
M3:3 Studies of cancer in experimental animals	M3 Diazinon	Gloria M. Calaf
M3:4.1 Toxicokinetic data	M3 Diazinon	Matt Ross
M3:4.2.1 Genetic and related effects	M3 Diazinon	Frank LeCurieux

Vol 112 – Overview of assignments

Section Title	Home Section	Author
M3:4.2.2 Receptor-mediated effects	M3 Diazinon	Lauren Zeise
M3:4.2.3 Oxidative stress, inflammation and immunosuppression	M3 Diazinon	Ivan Rusyn
M3:4.2.4 Altered cell proliferation	M3 Diazinon	Lauren Zeise
M3:4.2.5 Other mechanisms	M3 Diazinon	Lauren Zeise
M3:4.3 Data relevant to comparisons across agents and endpoints	M3 Diazinon	Matt Martin
M3:4.4 Cancer susceptibility data	M3 Diazinon	Ivan Rusyn
M3:4.5 Other adverse effects	M3 Diazinon	Matt Martin
M3:4.6 Mechanistic considerations	M3 Diazinon	Lauren Zeise
M4:1.1 Chemical and physical data	M4 Glyphosate	Peter P. Egeghy
M4:1.2 Production and use	M4 Glyphosate	Peter P. Egeghy
M4:1.3 Measurement and analysis	M4 Glyphosate	Peter P. Egeghy
M4:1.4 Occurrence and exposure	M4 Glyphosate	Teresa Rodriguez
M4:1.5 Regulations and guidelines	M4 Glyphosate	Hans Kromhout
M4:2 Studies of cancer in humans	M4 Glyphosate	Francesco Forastiere
M4:3 Studies of cancer in experimental animals	M4 Glyphosate	Charles (Bill) William Jameson
M4:4.1 Toxicokinetic data	M4 Glyphosate	Matt Ross
M4:4.2.1 Genetic and related effects	M4 Glyphosate	Frank LeCurieux
M4:4.2.2 Receptor-mediated effects	M4 Glyphosate	Lauren Zeise
M4:4.2.3Oxidative stress, inflammation and immunosuppression	M4 Glyphosate	Ivan Rusyn
M4:4.2.4 Altered cell proliferation	M4 Glyphosate	Lauren Zeise
M4:4.2.5Other mechanisms	M4 Glyphosate	Lauren Zeise
M4:4.3 Data relevant to comparisons across agents and endpoints	M4 Glyphosate	Matt Martin
M4:4.4Cancer susceptibility data	M4 Glyphosate	Ivan Rusyn
M4:4.5Other adverse effects	M4 Glyphosate	Matt Martin
M4:4.6 Mechanistic considerations	M4 Glyphosate	Ivan Rusyn
M5:1.1 Chemical and physical data	M5 Tetrachlorvinphos	Peter P. Egeghy
M5:1.2 Production and use	M5 Tetrachlorvinphos	Peter P. Egeghy
M5:1.3 Measurement and analysis	M5 Tetrachlorvinphos	Peter P. Egeghy
M5:1.4 Occurrence and exposure	M5 Tetrachlorvinphos	Teresa Rodriguez
M5:1.5 Regulations and guidelines	M5 Tetrachlorvinphos	Hans Kromhout
M5:2 Studies of cancer in humans	M5 Tetrachlorvinphos	Aaron Blair
M5:3 Studies of cancer in experimental animals	M5 Tetrachlorvinphos	Charles (Bill) William Jameson
M5:4.1 Toxicokinetic data	M5 Tetrachlorvinphos	Matt Ross
M5:4.2.1 Genetic and related effects	M5 Tetrachlorvinphos	Frank LeCurieux
M5:4.2.2 Receptor-mediated effects	M5 Tetrachlorvinphos	Lauren Zeise
M5:4.2.3 Other mechanisms	M5 Tetrachlorvinphos	Lauren Zeise
M5:4.3 Data relevant to comparisons across agents and endpoints	M5 Tetrachlorvinphos	Ivan Rusyn
M5:4.4 Cancer susceptibility data	M5 Tetrachlorvinphos	Ivan Rusyn
M5:4.5 Other adverse effects	M5 Tetrachlorvinphos	Matt Martin
M5:4.6 Mechanistic considerations	M5 Tetrachlorvinphos	Frank LeCurieux

Last update 11/20/2014

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IARC Monographs on the Evaluation of Carcinogenic Risks to Humans
VOLUME 112
IARC, Lyon, 3-10 March 2015

MEETING TIMETABLE

Monday, 2 March
15h30 – 17h00 Planning meeting – Meeting Chairs and subgroup Chairs only (rm 101, 1st floor)

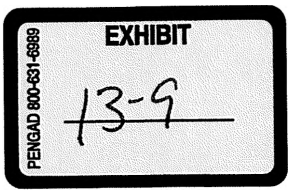
Tuesday, 3 March
09h00 – 09h30 Registration (Lobby)
09h30 – 10h30 Opening session: Director's welcome, introductions, programme overview
10h30 – 11h00 Group photo (Lobby, followed by coffee break)
11h00 – 13h00 Subgroup sessions
14h00 – 15h45 Subgroup sessions
15h45 – 16h15 Payment of *per diem* & dinner reservation (Lobby, during coffee break)
16h15 – 17h45 Subgroup sessions
17h45 – Cocktail reception for participants and their guests (12th floor)
18h15 – 19h00 Co-ordination meeting for the Co-chairs and subgroup Chairs (1st floor)

Wednesday, 4 March
09h00 – 09h30 Plenary session: Evaluation criteria
09h30 – 13h00 Subgroup sessions
14h00 – 18h00 Subgroup sessions
18h00 – 19h00 Co-ordination meeting for the Co-chairs and subgroup Chairs (1st floor)

Thursday, 5 March
09h00 – 09h10 Plenary session: Progress report
09h10 – 13h00 Subgroup sessions
14h00 – 15h45 Subgroup sessions
16h15 – 18h00 Subgroup sessions
18h00 – 19h00 Co-ordination meeting for the Co-chairs and subgroup Chairs (1st floor)

Friday, 6 March
09h00 – 09h10 Plenary session: Progress report
09h10 – 13h00 Subgroup sessions
14h00 – 15h45 Subgroup sessions
16h15 – 18h00 Plenary session: Overview discussion
18h00 – 19h00 Co-ordination meeting for the Co-chairs and subgroup Chairs (1st floor)

Saturday, 7 March
09h00 – 10h30 Subgroup sessions
11h00 – 15h00 Plenary session
20h00 Group dinner for participants and their guests



Monday, 9 March
09h00 – 13h00 Plenary session
14h00 – 18h00 Plenary session

Tuesday, 10 March
09h00 – 13h00 Plenary session
14h00 – 18h00 Plenary session
18h00 Adjourn

Lunch will be served on the 12th floor each day at 13h00 (12h30 on Saturday).
Coffee will be served in the lobby each day at 10h30 and 15h45.

WORLD HEALTH ORGANIZATION
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans

PREAMBLE

LYON, FRANCE
2006

CONTENTS

Amended January 2006

Last update September 2015

PREAMBLE

The Preamble to the *IARC Monographs* describes the objective and scope of the programme, the scientific principles and procedures used in developing a *Monograph*, the types of evidence considered and the scientific criteria that guide the evaluations. The Preamble should be consulted when reading a *Monograph* or list of evaluations.

A. GENERAL PRINCIPLES AND PROCEDURES

1. Background

Soon after IARC was established in 1965, it received frequent requests for advice on the carcinogenic risk of chemicals, including requests for lists of known and suspected human carcinogens. It was clear that it would not be a simple task to summarize adequately the complexity of the information that was available, and IARC began to consider means of obtaining international expert opinion on this topic. In 1970, the IARC Advisory Committee on Environmental Carcinogenesis recommended ' . . . that a compendium on carcinogenic chemicals be prepared by experts. The biological activity and evaluation of practical importance to public health should be referenced and documented.' The IARC Governing Council adopted a resolution concerning the role of IARC in providing government authorities with expert, independent, scientific opinion on environmental carcinogenesis. As one means to that end, the Governing Council recommended that IARC should prepare monographs on the evaluation of carcinogenic risk of chemicals to man, which became the initial title of the series.

In the succeeding years, the scope of the programme broadened as *Monographs* were developed for groups of related chemicals, complex mixtures, occupational exposures, physical and biological agents and lifestyle factors. In 1988, the phrase 'of chemicals' was dropped from the title, which assumed its present form, *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*.

Through the *Monographs* programme, IARC seeks to identify the causes of human cancer. This is the first step in cancer prevention, which is needed as much today as when IARC was established. The global burden of cancer is high and continues to increase: the annual number of new cases was estimated at 10.1 million in 2000 and is expected to reach 15 million by 2020 (Stewart & Kleihues, 2003). With current trends in demographics and exposure, the cancer burden has been shifting from high-resource countries to low- and medium-resource countries. As a result of *Monographs* evaluations, national health agencies have been able, on scientific grounds, to take measures to reduce human exposure to carcinogens in the workplace and in the environment.

The criteria established in 1971 to evaluate carcinogenic risks to humans were adopted by the Working Groups whose deliberations resulted in the first 16 volumes of the *Monographs* series. Those criteria were subsequently updated by further ad-hoc Advisory Groups (IARC, 1977, 1978, 1979, 1982, 1983, 1987, 1988, 1991; Vainio *et al.*, 1992; IARC, 2005, 2006).

The Preamble is primarily a statement of scientific principles, rather than a specification of working procedures. The procedures through which a Working Group implements these principles are not specified in detail. They usually involve operations that have been

established as being effective during previous *Monograph* meetings but remain, predominantly, the prerogative of each individual Working Group.

2. Objective and scope

The objective of the programme is to prepare, with the help of international Working Groups of experts, and to publish in the form of *Monographs*, critical reviews and evaluations of evidence on the carcinogenicity of a wide range of human exposures. The *Monographs* represent the first step in carcinogen risk assessment, which involves examination of all relevant information in order to assess the strength of the available evidence that an agent could alter the age-specific incidence of cancer in humans. The *Monographs* may also indicate where additional research efforts are needed, specifically when data immediately relevant to an evaluation are not available.

In this Preamble, the term 'agent' refers to any entity or circumstance that is subject to evaluation in a *Monograph*. As the scope of the programme has broadened, categories of agents now include specific chemicals, groups of related chemicals, complex mixtures, occupational or environmental exposures, cultural or behavioural practices, biological organisms and physical agents. This list of categories may expand as causation of, and susceptibility to, malignant disease become more fully understood.

A cancer 'hazard' is an agent that is capable of causing cancer under some circumstances, while a cancer 'risk' is an estimate of the carcinogenic effects expected from exposure to a cancer hazard. The *Monographs* are an exercise in evaluating cancer hazards, despite the historical presence of the word 'risks' in the title. The distinction between hazard and risk is important, and the *Monographs* identify cancer hazards even when risks are very low at current exposure levels, because new uses or unforeseen exposures could engender risks that are significantly higher.

In the *Monographs*, an agent is termed 'carcinogenic' if it is capable of increasing the incidence of malignant neoplasms, reducing their latency, or increasing their severity or multiplicity. The induction of benign neoplasms may in some circumstances (see Part B, Section 3a) contribute to the judgement that the agent is carcinogenic. The terms 'neoplasm' and 'tumour' are used interchangeably.

The Preamble continues the previous usage of the phrase 'strength of evidence' as a matter of historical continuity, although it should be understood that *Monographs* evaluations consider studies that support a finding of a cancer hazard as well as studies that do not.

Some epidemiological and experimental studies indicate that different agents may act at different stages in the carcinogenic process, and several different mechanisms may be involved. The aim of the *Monographs* has been, from their inception, to evaluate evidence of carcinogenicity at any stage in the carcinogenesis process, independently of the underlying mechanisms. Information on mechanisms may, however, be used in making the overall evaluation (IARC, 1991; Vainio *et al.*, 1992; IARC, 2005, 2006; see also Part B, Sections 4 and 6). As mechanisms of carcinogenesis are elucidated, IARC convenes international scientific conferences to determine whether a broad-based consensus has emerged on how specific mechanistic data can be used in an evaluation of human carcinogenicity. The results of such conferences are reported in IARC Scientific Publications, which, as long as they still reflect the current state of scientific knowledge, may guide subsequent Working Groups.

Although the *Monographs* have emphasized hazard identification, important issues may also involve dose–response assessment. In many cases, the same epidemiological and experimental studies used to evaluate a cancer hazard can also be used to estimate a dose–

response relationship. A *Monograph* may undertake to estimate dose–response relationships within the range of the available epidemiological data, or it may compare the dose–response information from experimental and epidemiological studies. In some cases, a subsequent publication may be prepared by a separate Working Group with expertise in quantitative dose–response assessment.

The *Monographs* are used by national and international authorities to make risk assessments, formulate decisions concerning preventive measures, provide effective cancer control programmes and decide among alternative options for public health decisions. The evaluations of IARC Working Groups are scientific, qualitative judgements on the evidence for or against carcinogenicity provided by the available data. These evaluations represent only one part of the body of information on which public health decisions may be based. Public health options vary from one situation to another and from country to country and relate to many factors, including different socioeconomic and national priorities. Therefore, no recommendation is given with regard to regulation or legislation, which are the responsibility of individual governments or other international organizations.

3. Selection of agents for review

Agents are selected for review on the basis of two main criteria: (a) there is evidence of human exposure and (b) there is some evidence or suspicion of carcinogenicity. Mixed exposures may occur in occupational and environmental settings and as a result of individual and cultural habits (such as tobacco smoking and dietary practices). Chemical analogues and compounds with biological or physical characteristics similar to those of suspected carcinogens may also be considered, even in the absence of data on a possible carcinogenic effect in humans or experimental animals.

The scientific literature is surveyed for published data relevant to an assessment of carcinogenicity. Ad-hoc Advisory Groups convened by IARC in 1984, 1989, 1991, 1993, 1998 and 2003 made recommendations as to which agents should be evaluated in the *Monographs* series. Recent recommendations are available on the *Monographs* programme website (http://monographs.iarc.fr). IARC may schedule other agents for review as it becomes aware of new scientific information or as national health agencies identify an urgent public health need related to cancer.

As significant new data become available on an agent for which a *Monograph* exists, a re-evaluation may be made at a subsequent meeting, and a new *Monograph* published. In some cases it may be appropriate to review only the data published since a prior evaluation. This can be useful for updating a database, reviewing new data to resolve a previously open question or identifying new tumour sites associated with a carcinogenic agent. Major changes in an evaluation (e.g. a new classification in Group 1 or a determination that a mechanism does not operate in humans, see Part B, Section 6) are more appropriately addressed by a full review.

4. Data for the *Monographs*

Each *Monograph* reviews all pertinent epidemiological studies and cancer bioassays in experimental animals. Those judged inadequate or irrelevant to the evaluation may be cited but not summarized. If a group of similar studies is not reviewed, the reasons are indicated.

Mechanistic and other relevant data are also reviewed. A *Monograph* does not necessarily cite all the mechanistic literature concerning the agent being evaluated (see Part B, Section

4). Only those data considered by the Working Group to be relevant to making the evaluation are included.

With regard to epidemiological studies, cancer bioassays, and mechanistic and other relevant data, only reports that have been published or accepted for publication in the openly available scientific literature are reviewed. The same publication requirement applies to studies originating from IARC, including meta-analyses or pooled analyses commissioned by IARC in advance of a meeting (see Part B, Section 2c). Data from government agency reports that are publicly available are also considered. Exceptionally, doctoral theses and other material that are in their final form and publicly available may be reviewed.

Exposure data and other information on an agent under consideration are also reviewed. In the sections on chemical and physical properties, on analysis, on production and use and on occurrence, published and unpublished sources of information may be considered.

Inclusion of a study does not imply acceptance of the adequacy of the study design or of the analysis and interpretation of the results, and limitations are clearly outlined in square brackets at the end of each study description (see Part B). The reasons for not giving further consideration to an individual study also are indicated in the square brackets.

5. Meeting participants

Five categories of participant can be present at *Monograph* meetings.

(a) The Working Group is responsible for the critical reviews and evaluations that are developed during the meeting. The tasks of Working Group Members are: (i) to ascertain that all appropriate data have been collected; (ii) to select the data relevant for the evaluation on the basis of scientific merit; (iii) to prepare accurate summaries of the data to enable the reader to follow the reasoning of the Working Group; (iv) to evaluate the results of epidemiological and experimental studies on cancer; (v) to evaluate data relevant to the understanding of mechanisms of carcinogenesis; and (vi) to make an overall evaluation of the carcinogenicity of the exposure to humans. Working Group Members generally have published significant research related to the carcinogenicity of the agents being reviewed, and IARC uses literature searches to identify most experts. Working Group Members are selected on the basis of (a) knowledge and experience and (b) absence of real or apparent conflicts of interests. Consideration is also given to demographic diversity and balance of scientific findings and views.

(b) Invited Specialists are experts who also have critical knowledge and experience but have a real or apparent conflict of interests. These experts are invited when necessary to assist in the Working Group by contributing their unique knowledge and experience during subgroup and plenary discussions. They may also contribute text on non-influential issues in the section on exposure, such as a general description of data on production and use (see Part B, Section 1). Invited Specialists do not serve as meeting chair or subgroup chair, draft text that pertains to the description or interpretation of cancer data, or participate in the evaluations.

(c) Representatives of national and international health agencies often attend meetings because their agencies sponsor the programme or are interested in the subject of a meeting. Representatives do not serve as meeting chair or subgroup chair, draft any part of a *Monograph*, or participate in the evaluations.

(d) Observers with relevant scientific credentials may be admitted to a meeting by IARC in limited numbers. Attention will be given to achieving a balance of Observers from constituencies with differing perspectives. They are invited to observe the meeting and

should not attempt to influence it. Observers do not serve as meeting chair or subgroup chair, draft any part of a *Monograph*, or participate in the evaluations. At the meeting, the meeting chair and subgroup chairs may grant Observers an opportunity to speak, generally after they have observed a discussion. Observers agree to respect the Guidelines for Observers at *IARC Monographs* meetings (available at http://monographs.iarc.fr).

(e) The IARC Secretariat consists of scientists who are designated by IARC and who have relevant expertise. They serve as rapporteurs and participate in all discussions. When requested by the meeting chair or subgroup chair, they may also draft text or prepare tables and analyses.

Before an invitation is extended, each potential participant, including the IARC Secretariat, completes the WHO Declaration of Interests to report financial interests, employment and consulting, and individual and institutional research support related to the subject of the meeting. IARC assesses these interests to determine whether there is a conflict that warrants some limitation on participation. The declarations are updated and reviewed again at the opening of the meeting. Interests related to the subject of the meeting are disclosed to the meeting participants and in the published volume (Cogliano *et al.*, 2004).

The names and principal affiliations of participants are available on the *Monographs* programme website (http://monographs.iarc.fr) approximately two months before each meeting. It is not acceptable for Observers or third parties to contact other participants before a meeting or to lobby them at any time. Meeting participants are asked to report all such contacts to IARC (Cogliano *et al.*, 2005).

All participants are listed, with their principal affiliations, at the beginning of each volume. Each participant who is a Member of a Working Group serves as an individual scientist and not as a representative of any organization, government or industry.

6. Working procedures

A separate Working Group is responsible for developing each volume of *Monographs*. A volume contains one or more *Monographs*, which can cover either a single agent or several related agents. Approximately one year in advance of the meeting of a Working Group, the agents to be reviewed are announced on the *Monographs* programme website (http://monographs.iarc.fr) and participants are selected by IARC staff in consultation with other experts. Subsequently, relevant biological and epidemiological data are collected by IARC from recognized sources of information on carcinogenesis, including data storage and retrieval systems such as PubMed. Meeting participants who are asked to prepare preliminary working papers for specific sections are expected to supplement the IARC literature searches with their own searches.

Industrial associations, labour unions and other knowledgeable organizations may be asked to provide input to the sections on production and use, although this involvement is not required as a general rule. Information on production and trade is obtained from governmental, trade and market research publications and, in some cases, by direct contact with industries. Separate production data on some agents may not be available for a variety of reasons (e.g. not collected or made public in all producing countries, production is small). Information on uses may be obtained from published sources but is often complemented by direct contact with manufacturers. Efforts are made to supplement this information with data from other national and international sources.

Six months before the meeting, the material obtained is sent to meeting participants to prepare preliminary working papers. The working papers are compiled by IARC staff and sent, prior to the meeting, to Working Group Members and Invited Specialists for review.

The Working Group meets at IARC for seven to eight days to discuss and finalize the texts and to formulate the evaluations. The objectives of the meeting are peer review and consensus. During the first few days, four subgroups (covering exposure data, cancer in humans, cancer in experimental animals, and mechanistic and other relevant data) review the working papers, develop a joint subgroup draft and write summaries. Care is taken to ensure that each study summary is written or reviewed by someone not associated with the study being considered. During the last few days, the Working Group meets in plenary session to review the subgroup drafts and develop the evaluations. As a result, the entire volume is the joint product of the Working Group, and there are no individually authored sections.

IARC Working Groups strive to achieve a consensus evaluation. Consensus reflects broad agreement among Working Group Members, but not necessarily unanimity. The chair may elect to poll Working Group Members to determine the diversity of scientific opinion on issues where consensus is not readily apparent.

After the meeting, the master copy is verified by consulting the original literature, edited and prepared for publication. The aim is to publish the volume within six months of the Working Group meeting. A summary of the outcome is available on the *Monographs* programme website soon after the meeting.

B. SCIENTIFIC REVIEW AND EVALUATION

The available studies are summarized by the Working Group, with particular regard to the qualitative aspects discussed below. In general, numerical findings are indicated as they appear in the original report; units are converted when necessary for easier comparison. The Working Group may conduct additional analyses of the published data and use them in their assessment of the evidence; the results of such supplementary analyses are given in square brackets. When an important aspect of a study that directly impinges on its interpretation should be brought to the attention of the reader, a Working Group comment is given in square brackets.

The scope of the *IARC Monographs* programme has expanded beyond chemicals to include complex mixtures, occupational exposures, physical and biological agents, lifestyle factors and other potentially carcinogenic exposures. Over time, the structure of a *Monograph* has evolved to include the following sections:

1. Exposure data
2. Studies of cancer in humans
3. Studies of cancer in experimental animals
4. Mechanistic and other relevant data
5. Summary
6. Evaluation and rationale

In addition, a section of General Remarks at the front of the volume discusses the reasons the agents were scheduled for evaluation and some key issues the Working Group encountered during the meeting.

This part of the Preamble discusses the types of evidence considered and summarized in each section of a *Monograph*, followed by the scientific criteria that guide the evaluations.

1. Exposure data

Each *Monograph* includes general information on the agent: this information may vary substantially between agents and must be adapted accordingly. Also included is information on production and use (when appropriate), methods of analysis and detection, occurrence, and sources and routes of human occupational and environmental exposures. Depending on the agent, regulations and guidelines for use may be presented.

(a) General information on the agent

For chemical agents, sections on chemical and physical data are included: the Chemical Abstracts Service Registry Number, the latest primary name and the IUPAC systematic name are recorded; other synonyms are given, but the list is not necessarily comprehensive. Information on chemical and physical properties that are relevant to identification, occurrence and biological activity is included. A description of technical products of chemicals includes trade names, relevant specifications and available information on composition and impurities. Some of the trade names given may be those of mixtures in which the agent being evaluated is only one of the ingredients.

For biological agents, taxonomy, structure and biology are described, and the degree of variability is indicated. Mode of replication, life cycle, target cells, persistence, latency, host response and clinical disease other than cancer are also presented.

For physical agents that are forms of radiation, energy and range of the radiation are included. For foreign bodies, fibres and respirable particles, size range and relative dimensions are indicated.

For agents such as mixtures, drugs or lifestyle factors, a description of the agent, including its composition, is given.

Whenever appropriate, other information, such as historical perspectives or the description of an industry or habit, may be included.

(b) Analysis and detection

An overview of methods of analysis and detection of the agent is presented, including their sensitivity, specificity and reproducibility. Methods widely used for regulatory purposes are emphasized. Methods for monitoring human exposure are also given. No critical evaluation or recommendation of any method is meant or implied.

(c) Production and use

The dates of first synthesis and of first commercial production of a chemical, mixture or other agent are provided when available; for agents that do not occur naturally, this information may allow a reasonable estimate to be made of the date before which no human exposure to the agent could have occurred. The dates of first reported occurrence of an exposure are also provided when available. In addition, methods of synthesis used in past and present commercial production and different methods of production, which may give rise to different impurities, are described.

The countries where companies report production of the agent, and the number of companies in each country, are identified. Available data on production, international trade and uses are obtained for representative regions. It should not, however, be inferred that those areas or nations are necessarily the sole or major sources or users of the agent. Some identified uses may not be current or major applications, and the coverage is not necessarily

comprehensive. In the case of drugs, mention of their therapeutic uses does not necessarily represent current practice nor does it imply judgement as to their therapeutic efficacy.

(d) Occurrence and exposure

Information on the occurrence of an agent in the environment is obtained from data derived from the monitoring and surveillance of levels in occupational environments, air, water, soil, plants, foods and animal and human tissues. When available, data on the generation, persistence and bioaccumulation of the agent are also included. Such data may be available from national databases.

Data that indicate the extent of past and present human exposure, the sources of exposure, the people most likely to be exposed and the factors that contribute to the exposure are reported. Information is presented on the range of human exposure, including occupational and environmental exposures. This includes relevant findings from both developed and developing countries. Some of these data are not distributed widely and may be available from government reports and other sources. In the case of mixtures, industries, occupations or processes, information is given about all agents known to be present. For processes, industries and occupations, a historical description is also given, noting variations in chemical composition, physical properties and levels of occupational exposure with date and place. For biological agents, the epidemiology of infection is described.

(e) Regulations and guidelines

Statements concerning regulations and guidelines (e.g. occupational exposure limits, maximal levels permitted in foods and water, pesticide registrations) are included, but they may not reflect the most recent situation, since such limits are continuously reviewed and modified. The absence of information on regulatory status for a country should not be taken to imply that that country does not have regulations with regard to the exposure. For biological agents, legislation and control, including vaccination and therapy, are described.

2. Studies of cancer in humans

This section includes all pertinent epidemiological studies (see Part A, Section 4). Studies of biomarkers are included when they are relevant to an evaluation of carcinogenicity to humans.

(a) Types of study considered

Several types of epidemiological study contribute to the assessment of carcinogenicity in humans — cohort studies, case–control studies, correlation (or ecological) studies and intervention studies. Rarely, results from randomized trials may be available. Case reports and case series of cancer in humans may also be reviewed.

Cohort and case–control studies relate individual exposures under study to the occurrence of cancer in individuals and provide an estimate of effect (such as relative risk) as the main measure of association. Intervention studies may provide strong evidence for making causal inferences, as exemplified by cessation of smoking and the subsequent decrease in risk for lung cancer.

In correlation studies, the units of investigation are usually whole populations (e.g. in particular geographical areas or at particular times), and cancer frequency is related to a summary measure of the exposure of the population to the agent under study. In correlation studies, individual exposure is not documented, which renders this kind of study more prone

to confounding. In some circumstances, however, correlation studies may be more informative than analytical study designs (see, for example, the *Monograph* on arsenic in drinking-water; IARC, 2004).

In some instances, case reports and case series have provided important information about the carcinogenicity of an agent. These types of study generally arise from a suspicion, based on clinical experience, that the concurrence of two events — that is, a particular exposure and occurrence of a cancer — has happened rather more frequently than would be expected by chance. Case reports and case series usually lack complete ascertainment of cases in any population, definition or enumeration of the population at risk and estimation of the expected number of cases in the absence of exposure.

The uncertainties that surround the interpretation of case reports, case series and correlation studies make them inadequate, except in rare instances, to form the sole basis for inferring a causal relationship. When taken together with case–control and cohort studies, however, these types of study may add materially to the judgement that a causal relationship exists.

Epidemiological studies of benign neoplasms, presumed preneoplastic lesions and other end-points thought to be relevant to cancer are also reviewed. They may, in some instances, strengthen inferences drawn from studies of cancer itself.

(b) Quality of studies considered

It is necessary to take into account the possible roles of bias, confounding and chance in the interpretation of epidemiological studies. Bias is the effect of factors in study design or execution that lead erroneously to a stronger or weaker association than in fact exists between an agent and disease. Confounding is a form of bias that occurs when the relationship with disease is made to appear stronger or weaker than it truly is as a result of an association between the apparent causal factor and another factor that is associated with either an increase or decrease in the incidence of the disease. The role of chance is related to biological variability and the influence of sample size on the precision of estimates of effect.

In evaluating the extent to which these factors have been minimized in an individual study, consideration is given to a number of aspects of design and analysis as described in the report of the study. For example, when suspicion of carcinogenicity arises largely from a single small study, careful consideration is given when interpreting subsequent studies that included these data in an enlarged population. Most of these considerations apply equally to case–control, cohort and correlation studies. Lack of clarity of any of these aspects in the reporting of a study can decrease its credibility and the weight given to it in the final evaluation of the exposure.

Firstly, the study population, disease (or diseases) and exposure should have been well defined by the authors. Cases of disease in the study population should have been identified in a way that was independent of the exposure of interest, and exposure should have been assessed in a way that was not related to disease status.

Secondly, the authors should have taken into account — in the study design and analysis — other variables that can influence the risk of disease and may have been related to the exposure of interest. Potential confounding by such variables should have been dealt with either in the design of the study, such as by matching, or in the analysis, by statistical adjustment. In cohort studies, comparisons with local rates of disease may or may not be more appropriate than those with national rates. Internal comparisons of frequency of disease among individuals at different levels of exposure are also desirable in cohort studies, since

they minimize the potential for confounding related to the difference in risk factors between an external reference group and the study population.

Thirdly, the authors should have reported the basic data on which the conclusions are founded, even if sophisticated statistical analyses were employed. At the very least, they should have given the numbers of exposed and unexposed cases and controls in a case–control study and the numbers of cases observed and expected in a cohort study. Further tabulations by time since exposure began and other temporal factors are also important. In a cohort study, data on all cancer sites and all causes of death should have been given, to reveal the possibility of reporting bias. In a case–control study, the effects of investigated factors other than the exposure of interest should have been reported.

Finally, the statistical methods used to obtain estimates of relative risk, absolute rates of cancer, confidence intervals and significance tests, and to adjust for confounding should have been clearly stated by the authors. These methods have been reviewed for case–control studies (Breslow & Day, 1980) and for cohort studies (Breslow & Day, 1987).

(c) Meta-analyses and pooled analyses

Independent epidemiological studies of the same agent may lead to results that are difficult to interpret. Combined analyses of data from multiple studies are a means of resolving this ambiguity, and well-conducted analyses can be considered. There are two types of combined analysis. The first involves combining summary statistics such as relative risks from individual studies (meta-analysis) and the second involves a pooled analysis of the raw data from the individual studies (pooled analysis) (Greenland, 1998).

The advantages of combined analyses are increased precision due to increased sample size and the opportunity to explore potential confounders, interactions and modifying effects that may explain heterogeneity among studies in more detail. A disadvantage of combined analyses is the possible lack of compatibility of data from various studies due to differences in subject recruitment, procedures of data collection, methods of measurement and effects of unmeasured co-variates that may differ among studies. Despite these limitations, well-conducted combined analyses may provide a firmer basis than individual studies for drawing conclusions about the potential carcinogenicity of agents.

IARC may commission a meta-analysis or pooled analysis that is pertinent to a particular *Monograph* (see Part A, Section 4). Additionally, as a means of gaining insight from the results of multiple individual studies, ad-hoc calculations that combine data from different studies may be conducted by the Working Group during the course of a *Monograph* meeting. The results of such original calculations, which would be specified in the text by presentation in square brackets, might involve updates of previously conducted analyses that incorporate the results of more recent studies or de-novo analyses. Irrespective of the source of data for the meta-analyses and pooled analyses, it is important that the same criteria for data quality be applied as those that would be applied to individual studies and to ensure also that sources of heterogeneity between studies be taken into account.

(d) Temporal effects

Detailed analyses of both relative and absolute risks in relation to temporal variables, such as age at first exposure, time since first exposure, duration of exposure, cumulative exposure, peak exposure (when appropriate) and time since cessation of exposure, are reviewed and summarized when available. Analyses of temporal relationships may be useful in making causal inferences. In addition, such analyses may suggest whether a carcinogen

acts early or late in the process of carcinogenesis, although, at best, they allow only indirect inferences about mechanisms of carcinogenesis.

(e) Use of biomarkers in epidemiological studies

Biomarkers indicate molecular, cellular or other biological changes and are increasingly used in epidemiological studies for various purposes (IARC, 1991; Vainio *et al.*, 1992; Toniolo *et al.*, 1997; Vineis *et al.*, 1999; Buffler *et al.*, 2004). These may include evidence of exposure, of early effects, of cellular, tissue or organism responses, of individual susceptibility or host responses, and inference of a mechanism (see Part B, Section 4b). This is a rapidly evolving field that encompasses developments in genomics, epigenomics and other emerging technologies.

Molecular epidemiological data that identify associations between genetic polymorphisms and interindividual differences in susceptibility to the agent(s) being evaluated may contribute to the identification of carcinogenic hazards to humans. If the polymorphism has been demonstrated experimentally to modify the functional activity of the gene product in a manner that is consistent with increased susceptibility, these data may be useful in making causal inferences. Similarly, molecular epidemiological studies that measure cell functions, enzymes or metabolites that are thought to be the basis of susceptibility may provide evidence that reinforces biological plausibility. It should be noted, however, that when data on genetic susceptibility originate from multiple comparisons that arise from subgroup analyses, this can generate false-positive results and inconsistencies across studies, and such data therefore require careful evaluation. If the known phenotype of a genetic polymorphism can explain the carcinogenic mechanism of the agent being evaluated, data on this phenotype may be useful in making causal inferences.

(f) Criteria for causality

After the quality of individual epidemiological studies of cancer has been summarized and assessed, a judgement is made concerning the strength of evidence that the agent in question is carcinogenic to humans. In making its judgement, the Working Group considers several criteria for causality (Hill, 1965). A strong association (e.g. a large relative risk) is more likely to indicate causality than a weak association, although it is recognized that estimates of effect of small magnitude do not imply lack of causality and may be important if the disease or exposure is common. Associations that are replicated in several studies of the same design or that use different epidemiological approaches or under different circumstances of exposure are more likely to represent a causal relationship than isolated observations from single studies. If there are inconsistent results among investigations, possible reasons are sought (such as differences in exposure), and results of studies that are judged to be of high quality are given more weight than those of studies that are judged to be methodologically less sound.

If the risk increases with the exposure, this is considered to be a strong indication of causality, although the absence of a graded response is not necessarily evidence against a causal relationship. The demonstration of a decline in risk after cessation of or reduction in exposure in individuals or in whole populations also supports a causal interpretation of the findings.

A number of scenarios may increase confidence in a causal relationship. On the one hand, an agent may be specific in causing tumours at one site or of one morphological type. On the other, carcinogenicity may be evident through the causation of multiple tumour types. Temporality, precision of estimates of effect, biological plausibility and coherence of the

overall database are considered. Data on biomarkers may be employed in an assessment of the biological plausibility of epidemiological observations.

Although rarely available, results from randomized trials that show different rates of cancer among exposed and unexposed individuals provide particularly strong evidence for causality.

When several epidemiological studies show little or no indication of an association between an exposure and cancer, a judgement may be made that, in the aggregate, they show evidence of lack of carcinogenicity. Such a judgement requires firstly that the studies meet, to a sufficient degree, the standards of design and analysis described above. Specifically, the possibility that bias, confounding or misclassification of exposure or outcome could explain the observed results should be considered and excluded with reasonable certainty. In addition, all studies that are judged to be methodologically sound should (a) be consistent with an estimate of effect of unity for any observed level of exposure, (b) when considered together, provide a pooled estimate of relative risk that is at or near to unity, and (c) have a narrow confidence interval, due to sufficient population size. Moreover, no individual study nor the pooled results of all the studies should show any consistent tendency that the relative risk of cancer increases with increasing level of exposure. It is important to note that evidence of lack of carcinogenicity obtained from several epidemiological studies can apply only to the type(s) of cancer studied, to the dose levels reported, and to the intervals between first exposure and disease onset observed in these studies. Experience with human cancer indicates that the period from first exposure to the development of clinical cancer is sometimes longer than 20 years; latent periods substantially shorter than 30 years cannot provide evidence for lack of carcinogenicity.

3. Studies of cancer in experimental animals

All known human carcinogens that have been studied adequately for carcinogenicity in experimental animals have produced positive results in one or more animal species (Wilbourn *et al.*, 1986; Tomatis *et al.*, 1989). For several agents (e.g. aflatoxins, diethylstilbestrol, solar radiation, vinyl chloride), carcinogenicity in experimental animals was established or highly suspected before epidemiological studies confirmed their carcinogenicity in humans (Vainio *et al.*, 1995). Although this association cannot establish that all agents that cause cancer in experimental animals also cause cancer in humans, it is biologically plausible that agents for which there is *sufficient evidence of carcinogenicity* in experimental animals (see Part B, Section 6b) also present a carcinogenic hazard to humans. Accordingly, in the absence of additional scientific information, these agents are considered to pose a carcinogenic hazard to humans. Examples of additional scientific information are data that demonstrate that a given agent causes cancer in animals through a species-specific mechanism that does not operate in humans or data that demonstrate that the mechanism in experimental animals also operates in humans (see Part B, Section 6).

Consideration is given to all available long-term studies of cancer in experimental animals with the agent under review (see Part A, Section 4). In all experimental settings, the nature and extent of impurities or contaminants present in the agent being evaluated are given when available. Animal species, strain (including genetic background where applicable), sex, numbers per group, age at start of treatment, route of exposure, dose levels, duration of exposure, survival and information on tumours (incidence, latency, severity or multiplicity of neoplasms or preneoplastic lesions) are reported. Those studies in experimental animals that are judged to be irrelevant to the evaluation or judged to be inadequate (e.g. too short a

duration, too few animals, poor survival; see below) may be omitted. Guidelines for conducting long-term carcinogenicity experiments have been published (e.g. OECD, 2002).

Other studies considered may include: experiments in which the agent was administered in the presence of factors that modify carcinogenic effects (e.g. initiation–promotion studies, co-carcinogenicity studies and studies in genetically modified animals); studies in which the end-point was not cancer but a defined precancerous lesion; experiments on the carcinogenicity of known metabolites and derivatives; and studies of cancer in non-laboratory animals (e.g. livestock and companion animals) exposed to the agent.

For studies of mixtures, consideration is given to the possibility that changes in the physicochemical properties of the individual substances may occur during collection, storage, extraction, concentration and delivery. Another consideration is that chemical and toxicological interactions of components in a mixture may alter dose–response relationships. The relevance to human exposure of the test mixture administered in the animal experiment is also assessed. This may involve consideration of the following aspects of the mixture tested: (i) physical and chemical characteristics, (ii) identified constituents that may indicate the presence of a class of substances and (iii) the results of genetic toxicity and related tests.

The relevance of results obtained with an agent that is analogous (e.g. similar in structure or of a similar virus genus) to that being evaluated is also considered. Such results may provide biological and mechanistic information that is relevant to the understanding of the process of carcinogenesis in humans and may strengthen the biological plausibility that the agent being evaluated is carcinogenic to humans (see Part B, Section 2f).

(a) Qualitative aspects

An assessment of carcinogenicity involves several considerations of qualitative importance, including (i) the experimental conditions under which the test was performed, including route, schedule and duration of exposure, species, strain (including genetic background where applicable), sex, age and duration of follow-up; (ii) the consistency of the results, for example, across species and target organ(s); (iii) the spectrum of neoplastic response, from preneoplastic lesions and benign tumours to malignant neoplasms; and (iv) the possible role of modifying factors.

Considerations of importance in the interpretation and evaluation of a particular study include: (i) how clearly the agent was defined and, in the case of mixtures, how adequately the sample characterization was reported; (ii) whether the dose was monitored adequately, particularly in inhalation experiments; (iii) whether the doses, duration of treatment and route of exposure were appropriate; (iv) whether the survival of treated animals was similar to that of controls; (v) whether there were adequate numbers of animals per group; (vi) whether both male and female animals were used; (vii) whether animals were allocated randomly to groups; (viii) whether the duration of observation was adequate; and (ix) whether the data were reported and analysed adequately.

When benign tumours (a) occur together with and originate from the same cell type as malignant tumours in an organ or tissue in a particular study and (b) appear to represent a stage in the progression to malignancy, they are usually combined in the assessment of tumour incidence (Huff *et al.*, 1989). The occurrence of lesions presumed to be preneoplastic may in certain instances aid in assessing the biological plausibility of any neoplastic response observed. If an agent induces only benign neoplasms that appear to be end-points that do not readily undergo transition to malignancy, the agent should nevertheless be suspected of being carcinogenic and requires further investigation.

(b) Quantitative aspects

The probability that tumours will occur may depend on the species, sex, strain, genetic background and age of the animal, and on the dose, route, timing and duration of the exposure. Evidence of an increased incidence of neoplasms with increasing levels of exposure strengthens the inference of a causal association between the exposure and the development of neoplasms.

The form of the dose–response relationship can vary widely, depending on the particular agent under study and the target organ. Mechanisms such as induction of DNA damage or inhibition of repair, altered cell division and cell death rates and changes in intercellular communication are important determinants of dose–response relationships for some carcinogens. Since many chemicals require metabolic activation before being converted to their reactive intermediates, both metabolic and toxicokinetic aspects are important in determining the dose–response pattern. Saturation of steps such as absorption, activation, inactivation and elimination may produce non-linearity in the dose–response relationship (Hoel *et al.*, 1983; Gart *et al.*, 1986), as could saturation of processes such as DNA repair. The dose–response relationship can also be affected by differences in survival among the treatment groups.

(c) Statistical analyses

Factors considered include the adequacy of the information given for each treatment group: (i) number of animals studied and number examined histologically, (ii) number of animals with a given tumour type and (iii) length of survival. The statistical methods used should be clearly stated and should be the generally accepted techniques refined for this purpose (Peto *et al.*, 1980; Gart *et al.*, 1986; Portier & Bailer, 1989; Bieler & Williams, 1993). The choice of the most appropriate statistical method requires consideration of whether or not there are differences in survival among the treatment groups; for example, reduced survival because of non-tumour-related mortality can preclude the occurrence of tumours later in life. When detailed information on survival is not available, comparisons of the proportions of tumour-bearing animals among the effective number of animals (alive at the time the first tumour was discovered) can be useful when significant differences in survival occur before tumours appear. The lethality of the tumour also requires consideration: for rapidly fatal tumours, the time of death provides an indication of the time of tumour onset and can be assessed using life-table methods; non-fatal or incidental tumours that do not affect survival can be assessed using methods such as the Mantel-Haenzel test for changes in tumour prevalence. Because tumour lethality is often difficult to determine, methods such as the Poly-K test that do not require such information can also be used. When results are available on the number and size of tumours seen in experimental animals (e.g. papillomas on mouse skin, liver tumours observed through nuclear magnetic resonance tomography), other more complicated statistical procedures may be needed (Sherman *et al.*, 1994; Dunson *et al.*, 2003).

Formal statistical methods have been developed to incorporate historical control data into the analysis of data from a given experiment. These methods assign an appropriate weight to historical and concurrent controls on the basis of the extent of between-study and within-study variability: less weight is given to historical controls when they show a high degree of variability, and greater weight when they show little variability. It is generally not appropriate to discount a tumour response that is significantly increased compared with concurrent controls by arguing that it falls within the range of historical controls, particularly when historical controls show high between-study variability and are, thus, of little relevance to the

current experiment. In analysing results for uncommon tumours, however, the analysis may be improved by considering historical control data, particularly when between-study variability is low. Historical controls should be selected to resemble the concurrent controls as closely as possible with respect to species, gender and strain, as well as other factors such as basal diet and general laboratory environment, which may affect tumour-response rates in control animals (Haseman *et al.*, 1984; Fung *et al.*, 1996; Greim *et al.*, 2003).

Although meta-analyses and combined analyses are conducted less frequently for animal experiments than for epidemiological studies due to differences in animal strains, they can be useful aids in interpreting animal data when the experimental protocols are sufficiently similar.

4. Mechanistic and other relevant data

Mechanistic and other relevant data may provide evidence of carcinogenicity and also help in assessing the relevance and importance of findings of cancer in animals and in humans. The nature of the mechanistic and other relevant data depends on the biological activity of the agent being considered. The Working Group considers representative studies to give a concise description of the relevant data and issues that they consider to be important; thus, not every available study is cited. Relevant topics may include toxicokinetics, mechanisms of carcinogenesis, susceptible individuals, populations and life-stages, other relevant data and other adverse effects. When data on biomarkers are informative about the mechanisms of carcinogenesis, they are included in this section.

These topics are not mutually exclusive; thus, the same studies may be discussed in more than one subsection. For example, a mutation in a gene that codes for an enzyme that metabolizes the agent under study could be discussed in the subsections on toxicokinetics, mechanisms and individual susceptibility if it also exists as an inherited polymorphism.

(a) Toxicokinetic data

Toxicokinetics refers to the absorption, distribution, metabolism and elimination of agents in humans, experimental animals and, where relevant, cellular systems. Examples of kinetic factors that may affect dose–response relationships include uptake, deposition, biopersistence and half-life in tissues, protein binding, metabolic activation and detoxification. Studies that indicate the metabolic fate of the agent in humans and in experimental animals are summarized briefly, and comparisons of data from humans and animals are made when possible. Comparative information on the relationship between exposure and the dose that reaches the target site may be important for the extrapolation of hazards between species and in clarifying the role of in-vitro findings.

(b) Data on mechanisms of carcinogenesis

To provide focus, the Working Group attempts to identify the possible mechanisms by which the agent may increase the risk of cancer. For each possible mechanism, a representative selection of key data from humans and experimental systems is summarized. Attention is given to gaps in the data and to data that suggests that more than one mechanism may be operating. The relevance of the mechanism to humans is discussed, in particular, when mechanistic data are derived from experimental model systems. Changes in the affected organs, tissues or cells can be divided into three non-exclusive levels as described below.

(i) Changes in physiology

Physiological changes refer to exposure-related modifications to the physiology and/or response of cells, tissues and organs. Examples of potentially adverse physiological changes include mitogenesis, compensatory cell division, escape from apoptosis and/or senescence, presence of inflammation, hyperplasia, metaplasia and/or preneoplasia, angiogenesis, alterations in cellular adhesion, changes in steroidal hormones and changes in immune surveillance.

(ii) Functional changes at the cellular level

Functional changes refer to exposure-related alterations in the signalling pathways used by cells to manage critical processes that are related to increased risk for cancer. Examples of functional changes include modified activities of enzymes involved in the metabolism of xenobiotics, alterations in the expression of key genes that regulate DNA repair, alterations in cyclin-dependent kinases that govern cell cycle progression, changes in the patterns of post-translational modifications of proteins, changes in regulatory factors that alter apoptotic rates, changes in the secretion of factors related to the stimulation of DNA replication and transcription and changes in gap–junction-mediated intercellular communication.

(iii) Changes at the molecular level

Molecular changes refer to exposure-related changes in key cellular structures at the molecular level, including, in particular, genotoxicity. Examples of molecular changes include formation of DNA adducts and DNA strand breaks, mutations in genes, chromosomal aberrations, aneuploidy and changes in DNA methylation patterns. Greater emphasis is given to irreversible effects.

The use of mechanistic data in the identification of a carcinogenic hazard is specific to the mechanism being addressed and is not readily described for every possible level and mechanism discussed above.

Genotoxicity data are discussed here to illustrate the key issues involved in the evaluation of mechanistic data.

Tests for genetic and related effects are described in view of the relevance of gene mutation and chromosomal aberration/aneuploidy to carcinogenesis (Vainio *et al.*, 1992; McGregor *et al.*, 1999). The adequacy of the reporting of sample characterization is considered and, when necessary, commented upon; with regard to complex mixtures, such comments are similar to those described for animal carcinogenicity tests. The available data are interpreted critically according to the end-points detected, which may include DNA damage, gene mutation, sister chromatid exchange, micronucleus formation, chromosomal aberrations and aneuploidy. The concentrations employed are given, and mention is made of whether the use of an exogenous metabolic system *in vitro* affected the test result. These data are listed in tabular form by phylogenetic classification.

Positive results in tests using prokaryotes, lower eukaryotes, insects, plants and cultured mammalian cells suggest that genetic and related effects could occur in mammals. Results from such tests may also give information on the types of genetic effect produced and on the involvement of metabolic activation. Some end-points described are clearly genetic in nature (e.g. gene mutations), while others are associated with genetic effects (e.g. unscheduled DNA synthesis). In-vitro tests for

tumour promotion, cell transformation and gap–junction intercellular communication may be sensitive to changes that are not necessarily the result of genetic alterations but that may have specific relevance to the process of carcinogenesis. Critical appraisals of these tests have been published (Montesano *et al.*, 1986; McGregor *et al.*, 1999).

Genetic or other activity manifest in humans and experimental mammals is regarded to be of greater relevance than that in other organisms. The demonstration that an agent can induce gene and chromosomal mutations in mammals *in vivo* indicates that it may have carcinogenic activity. Negative results in tests for mutagenicity in selected tissues from animals treated *in vivo* provide less weight, partly because they do not exclude the possibility of an effect in tissues other than those examined. Moreover, negative results in short-term tests with genetic end-points cannot be considered to provide evidence that rules out the carcinogenicity of agents that act through other mechanisms (e.g. receptor-mediated effects, cellular toxicity with regenerative cell division, peroxisome proliferation) (Vainio *et al.*, 1992). Factors that may give misleading results in short-term tests have been discussed in detail elsewhere (Montesano *et al.*, 1986; McGregor *et al.*, 1999).

When there is evidence that an agent acts by a specific mechanism that does not involve genotoxicity (e.g. hormonal dysregulation, immune suppression, and formation of calculi and other deposits that cause chronic irritation), that evidence is presented and reviewed critically in the context of rigorous criteria for the operation of that mechanism in carcinogenesis (e.g. Capen *et al.*, 1999).

For biological agents such as viruses, bacteria and parasites, other data relevant to carcinogenicity may include descriptions of the pathology of infection, integration and expression of viruses, and genetic alterations seen in human tumours. Other observations that might comprise cellular and tissue responses to infection, immune response and the presence of tumour markers are also considered.

For physical agents that are forms of radiation, other data relevant to carcinogenicity may include descriptions of damaging effects at the physiological, cellular and molecular level, as for chemical agents, and descriptions of how these effects occur. 'Physical agents' may also be considered to comprise foreign bodies, such as surgical implants of various kinds, and poorly soluble fibres, dusts and particles of various sizes, the pathogenic effects of which are a result of their physical presence in tissues or body cavities. Other relevant data for such materials may include characterization of cellular, tissue and physiological reactions to these materials and descriptions of pathological conditions other than neoplasia with which they may be associated.

(c) Other data relevant to mechanisms

A description is provided of any structure–activity relationships that may be relevant to an evaluation of the carcinogenicity of an agent, the toxicological implications of the physical and chemical properties, and any other data relevant to the evaluation that are not included elsewhere.

High-output data, such as those derived from gene expression microarrays, and high-throughput data, such as those that result from testing hundreds of agents for a single end-point, pose a unique problem for the use of mechanistic data in the evaluation of a carcinogenic hazard. In the case of high-output data, there is the possibility to overinterpret changes in individual end-points (e.g. changes in expression in one gene) without considering the consistency of that finding in the broader context of the other end-points (e.g. other genes

with linked transcriptional control). High-output data can be used in assessing mechanisms, but all end-points measured in a single experiment need to be considered in the proper context. For high-throughput data, where the number of observations far exceeds the number of end-points measured, their utility for identifying common mechanisms across multiple agents is enhanced. These data can be used to identify mechanisms that not only seem plausible, but also have a consistent pattern of carcinogenic response across entire classes of related compounds.

(d) Susceptibility data

Individuals, populations and life-stages may have greater or lesser susceptibility to an agent, based on toxicokinetics, mechanisms of carcinogenesis and other factors. Examples of host and genetic factors that affect individual susceptibility include sex, genetic polymorphisms of genes involved in the metabolism of the agent under evaluation, differences in metabolic capacity due to life-stage or the presence of disease, differences in DNA repair capacity, competition for or alteration of metabolic capacity by medications or other chemical exposures, pre-existing hormonal imbalance that is exacerbated by a chemical exposure, a suppressed immune system, periods of higher-than-usual tissue growth or regeneration and genetic polymorphisms that lead to differences in behaviour (e.g. addiction). Such data can substantially increase the strength of the evidence from epidemiological data and enhance the linkage of in-vivo and in-vitro laboratory studies to humans.

(e) Data on other adverse effects

Data on acute, subchronic and chronic adverse effects relevant to the cancer evaluation are summarized. Adverse effects that confirm distribution and biological effects at the sites of tumour development, or alterations in physiology that could lead to tumour development, are emphasized. Effects on reproduction, embryonic and fetal survival and development are summarized briefly. The adequacy of epidemiological studies of reproductive outcome and genetic and related effects in humans is judged by the same criteria as those applied to epidemiological studies of cancer, but fewer details are given.

5. Summary

This section is a summary of data presented in the preceding sections. Summaries can be found on the *Monographs* programme website (http://monographs.iarc.fr).

(a) Exposure data

Data are summarized, as appropriate, on the basis of elements such as production, use, occurrence and exposure levels in the workplace and environment and measurements in human tissues and body fluids. Quantitative data and time trends are given to compare exposures in different occupations and environmental settings. Exposure to biological agents is described in terms of transmission, prevalence and persistence of infection.

(b) Cancer in humans

Results of epidemiological studies pertinent to an assessment of human carcinogenicity are summarized. When relevant, case reports and correlation studies are also summarized. The target organ(s) or tissue(s) in which an increase in cancer was observed is identified. Dose–response and other quantitative data may be summarized when available.

(c) Cancer in experimental animals

Data relevant to an evaluation of carcinogenicity in animals are summarized. For each animal species, study design and route of administration, it is stated whether an increased incidence, reduced latency, or increased severity or multiplicity of neoplasms or preneoplastic lesions were observed, and the tumour sites are indicated. If the agent produced tumours after prenatal exposure or in single-dose experiments, this is also mentioned. Negative findings, inverse relationships, dose–response and other quantitative data are also summarized.

(d) Mechanistic and other relevant data

Data relevant to the toxicokinetics (absorption, distribution, metabolism, elimination) and the possible mechanism(s) of carcinogenesis (e.g. genetic toxicity, epigenetic effects) are summarized. In addition, information on susceptible individuals, populations and life-stages is summarized. This section also reports on other toxic effects, including reproductive and developmental effects, as well as additional relevant data that are considered to be important.

6. Evaluation and rationale

Evaluations of the strength of the evidence for carcinogenicity arising from human and experimental animal data are made, using standard terms. The strength of the mechanistic evidence is also characterized.

It is recognized that the criteria for these evaluations, described below, cannot encompass all of the factors that may be relevant to an evaluation of carcinogenicity. In considering all of the relevant scientific data, the Working Group may assign the agent to a higher or lower category than a strict interpretation of these criteria would indicate.

These categories refer only to the strength of the evidence that an exposure is carcinogenic and not to the extent of its carcinogenic activity (potency). A classification may change as new information becomes available.

An evaluation of the degree of evidence is limited to the materials tested, as defined physically, chemically or biologically. When the agents evaluated are considered by the Working Group to be sufficiently closely related, they may be grouped together for the purpose of a single evaluation of the degree of evidence.

(a) Carcinogenicity in humans

The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

Sufficient evidence of carcinogenicity: The Working Group considers that a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence. A statement that there is *sufficient evidence* is followed by a separate sentence that identifies the target organ(s) or tissue(s) where an increased risk of cancer was observed in humans. Identification of a specific target organ or tissue does not preclude the possibility that the agent may cause cancer at other sites.

Limited evidence of carcinogenicity: A positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered by the

Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.

Inadequate evidence of carcinogenicity: The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of a causal association between exposure and cancer, or no data on cancer in humans are available.

Evidence suggesting lack of carcinogenicity: There are several adequate studies covering the full range of levels of exposure that humans are known to encounter, which are mutually consistent in not showing a positive association between exposure to the agent and any studied cancer at any observed level of exposure. The results from these studies alone or combined should have narrow confidence intervals with an upper limit close to the null value (e.g. a relative risk of 1.0). Bias and confounding should be ruled out with reasonable confidence, and the studies should have an adequate length of follow-up. A conclusion of *evidence suggesting lack of carcinogenicity* is inevitably limited to the cancer sites, conditions and levels of exposure, and length of observation covered by the available studies. In addition, the possibility of a very small risk at the levels of exposure studied can never be excluded.

In some instances, the above categories may be used to classify the degree of evidence related to carcinogenicity in specific organs or tissues.

When the available epidemiological studies pertain to a mixture, process, occupation or industry, the Working Group seeks to identify the specific agent considered most likely to be responsible for any excess risk. The evaluation is focused as narrowly as the available data on exposure and other aspects permit.

(b) Carcinogenicity in experimental animals

Carcinogenicity in experimental animals can be evaluated using conventional bioassays, bioassays that employ genetically modified animals, and other in-vivo bioassays that focus on one or more of the critical stages of carcinogenesis. In the absence of data from conventional long-term bioassays or from assays with neoplasia as the end-point, consistently positive results in several models that address several stages in the multistage process of carcinogenesis should be considered in evaluating the degree of evidence of carcinogenicity in experimental animals.

The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories:

Sufficient evidence of carcinogenicity: The Working Group considers that a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide *sufficient evidence*.

A single study in one species and sex might be considered to provide *sufficient evidence of carcinogenicity* when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.

Limited evidence of carcinogenicity: The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.

Inadequate evidence of carcinogenicity: The studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect because of major qualitative or quantitative limitations, or no data on cancer in experimental animals are available.

Evidence suggesting lack of carcinogenicity: Adequate studies involving at least two species are available which show that, within the limits of the tests used, the agent is not carcinogenic. A conclusion of *evidence suggesting lack of carcinogenicity* is inevitably limited to the species, tumour sites, age at exposure, and conditions and levels of exposure studied.

(c) Mechanistic and other relevant data

Mechanistic and other evidence judged to be relevant to an evaluation of carcinogenicity and of sufficient importance to affect the overall evaluation is highlighted. This may include data on preneoplastic lesions, tumour pathology, genetic and related effects, structure–activity relationships, metabolism and toxicokinetics, physicochemical parameters and analogous biological agents.

The strength of the evidence that any carcinogenic effect observed is due to a particular mechanism is evaluated, using terms such as 'weak', 'moderate' or 'strong'. The Working Group then assesses whether that particular mechanism is likely to be operative in humans. The strongest indications that a particular mechanism operates in humans derive from data on humans or biological specimens obtained from exposed humans. The data may be considered to be especially relevant if they show that the agent in question has caused changes in exposed humans that are on the causal pathway to carcinogenesis. Such data may, however, never become available, because it is at least conceivable that certain compounds may be kept from human use solely on the basis of evidence of their toxicity and/or carcinogenicity in experimental systems.

The conclusion that a mechanism operates in experimental animals is strengthened by findings of consistent results in different experimental systems, by the demonstration of biological plausibility and by coherence of the overall database. Strong support can be obtained from studies that challenge the hypothesized mechanism experimentally, by demonstrating that the suppression of key mechanistic processes leads to the suppression of tumour development. The Working Group considers whether multiple mechanisms might contribute to tumour development, whether different mechanisms might operate in different dose ranges, whether separate mechanisms might operate in humans and experimental animals and whether a unique mechanism might operate in a susceptible group. The possible contribution of alternative mechanisms must be considered before concluding that tumours observed in experimental animals are not relevant to humans. An uneven level of experimental support for different mechanisms may reflect that disproportionate resources have been focused on investigating a favoured mechanism.

For complex exposures, including occupational and industrial exposures, the chemical composition and the potential contribution of carcinogens known to be present are considered by the Working Group in its overall evaluation of human carcinogenicity. The Working

Group also determines the extent to which the materials tested in experimental systems are related to those to which humans are exposed.

(d) Overall evaluation

Finally, the body of evidence is considered as a whole, in order to reach an overall evaluation of the carcinogenicity of the agent to humans.

An evaluation may be made for a group of agents that have been evaluated by the Working Group. In addition, when supporting data indicate that other related agents, for which there is no direct evidence of their capacity to induce cancer in humans or in animals, may also be carcinogenic, a statement describing the rationale for this conclusion is added to the evaluation narrative; an additional evaluation may be made for this broader group of agents if the strength of the evidence warrants it.

The agent is described according to the wording of one of the following categories, and the designated group is given. The categorization of an agent is a matter of scientific judgement that reflects the strength of the evidence derived from studies in humans and in experimental animals and from mechanistic and other relevant data.

Group 1: The agent is *carcinogenic to humans*.

This category is used when there is *sufficient evidence of carcinogenicity* in humans. Exceptionally, an agent may be placed in this category when evidence of carcinogenicity in humans is less than *sufficient* but there is *sufficient evidence of carcinogenicity* in experimental animals and strong evidence in exposed humans that the agent acts through a relevant mechanism of carcinogenicity.

Group 2.

This category includes agents for which, at one extreme, the degree of evidence of carcinogenicity in humans is almost *sufficient*, as well as those for which, at the other extreme, there are no human data but for which there is evidence of carcinogenicity in experimental animals. Agents are assigned to either Group 2A (*probably carcinogenic to humans*) or Group 2B (*possibly carcinogenic to humans*) on the basis of epidemiological and experimental evidence of carcinogenicity and mechanistic and other relevant data. The terms *probably carcinogenic* and *possibly carcinogenic* have no quantitative significance and are used simply as descriptors of different levels of evidence of human carcinogenicity, with *probably carcinogenic* signifying a higher level of evidence than *possibly carcinogenic*.

Group 2A: The agent is *probably carcinogenic to humans*.

This category is used when there is *limited evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals. In some cases, an agent may be classified in this category when there is *inadequate evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals and strong evidence that the carcinogenesis is mediated by a mechanism that also operates in humans. Exceptionally, an agent may be classified in this category solely on the basis of *limited evidence of carcinogenicity* in humans. An agent may be assigned to this category if it clearly belongs, based on mechanistic considerations, to a class of agents for which one or more members have been classified in Group 1 or Group 2A.

Group 2B: The agent is *possibly carcinogenic to humans.*

This category is used for agents for which there is *limited evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals. It may also be used when there is *inadequate evidence of carcinogenicity* in humans but there is *sufficient evidence of carcinogenicity* in experimental animals. In some instances, an agent for which there is *inadequate evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals together with supporting evidence from mechanistic and other relevant data may be placed in this group. An agent may be classified in this category solely on the basis of strong evidence from mechanistic and other relevant data.

Group 3: The agent is *not classifiable as to its carcinogenicity to humans.*

This category is used most commonly for agents for which the evidence of carcinogenicity is *inadequate* in humans and *inadequate* or *limited* in experimental animals.

Exceptionally, agents for which the evidence of carcinogenicity is *inadequate* in humans but *sufficient* in experimental animals may be placed in this category when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans.

Agents that do not fall into any other group are also placed in this category.

An evaluation in Group 3 is not a determination of non-carcinogenicity or overall safety. It often means that further research is needed, especially when exposures are widespread or the cancer data are consistent with differing interpretations.

Group 4: The agent is *probably not carcinogenic to humans.*

This category is used for agents for which there is *evidence suggesting lack of carcinogenicity* in humans and in experimental animals. In some instances, agents for which there is *inadequate evidence of carcinogenicity* in humans but *evidence suggesting lack of carcinogenicity* in experimental animals, consistently and strongly supported by a broad range of mechanistic and other relevant data, may be classified in this group.

(e) Rationale

The reasoning that the Working Group used to reach its evaluation is presented and discussed. This section integrates the major findings from studies of cancer in humans, studies of cancer in experimental animals, and mechanistic and other relevant data. It includes concise statements of the principal line(s) of argument that emerged, the conclusions of the Working Group on the strength of the evidence for each group of studies, citations to indicate which studies were pivotal to these conclusions, and an explanation of the reasoning of the Working Group in weighing data and making evaluations. When there are significant differences of scientific interpretation among Working Group Members, a brief summary of the alternative interpretations is provided, together with their scientific rationale and an indication of the relative degree of support for each alternative.

References

Bieler, G.S. & Williams, R.L. (1993) Ratio estimates, the delta method, and quantal response tests for increased carcinogenicity. *Biometrics*, 49(3), 793–801

Breslow, N.E. & Day, N.E. (1980) *Statistical Methods in Cancer Research*, Vol. 1, *The Analysis of Case-Control Studies* (IARC Scientific Publications No. 32), Lyon, IARC

Breslow, N.E. & Day, N.E. (1987) *Statistical Methods in Cancer Research*, Vol. 2, *The Design and Analysis of Cohort Studies* (IARC Scientific Publications No. 82), Lyon, IARC

Buffler, P., Rice, J., Baan, R., Bird, M. & Boffetta, P., eds (2004) *Mechanisms of Carcinogenesis* (IARC Scientific Publications No. 157), Lyon, IARC

Capen, C.C., Dybing, E., Rice, J.M. & Wilbourn, J.D. (1999) *Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis* (IARC Scientific Publications No. 147), Lyon, IARC

Cogliano, V.J., Baan, R.A., Straif, K., Grosse, Y., Secretan, M.B., El Ghissassi, F. & Kleihues, P. (2004) The science and practice of carcinogen identification and evaluation. *Environmental Health Perspect.*, 112(13), 1269–1274

Cogliano, V., Baan, R., Straif, K., Grosse, Y., Secretan, B., El Ghissassi, F. & Boyle, P. (2005) Transparency in IARC Monographs. *Lancet Oncol.*, 6(10), 747

Dunson, D.B., Chen, Z. & Harry, J. (2003) A Bayesian approach for joint modeling of cluster size and subunit-specific outcomes. *Biometrics*, 59(3), 521–30

Fung, K.Y., Krewski, D. & Smythe, R.T. (1996) A comparison of tests for trend with historical controls in carcinogen bioassay. *Can. J. Statist.*, 24, 431–454

Gart, J.J., Krewski, D., Lee, P.N., Tarone, R.E. & Wahrendorf, J. (1986) *Statistical Methods in Cancer Research*, Vol. 3, *The Design and Analysis of Long-term Animal Experiments* (IARC Scientific Publications No. 79), Lyon, IARC

Greenland, S. (1998) Meta-analysis. In: Rothman, K.J. & Greenland, S., eds, *Modern Epidemiology*, Philadelphia, Lippincott Williams & Wilkins, pp. 643–673

Greim, H., Gelbke, H.-P., Reuter, U., Thielmann, H.W. & Edler, L. (2003) Evaluation of historical control data in carcinogenicity studies. *Hum. exp. Toxicol.*, 22, 541–549

Haseman, J.K., Huff, J. & Boorman, G.A. (1984) Use of historical control data in carcinogenicity studies in rodents. *Toxicol. Pathol.*, 12(2), 126–135

Hill, A.B. (1965) The environment and disease: Association or causation? *Proc. R. Soc. Med.*, 58, 295–300

Hoel, D.G., Kaplan, N.L. & Anderson, M.W. (1983) Implication of nonlinear kinetics on risk estimation in carcinogenesis. *Science*, 219, 1032–1037

Huff, J.E., Eustis, S.L. & Haseman, J.K. (1989) Occurrence and relevance of chemically induced benign neoplasms in long-term carcinogenicity studies. *Cancer Metastasis Rev.*, 8, 1–21

IARC (1977) *IARC Monographs Programme on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*. Preamble (IARC intern. tech. Rep. No. 77/002)

IARC (1978) *Chemicals with Sufficient Evidence of Carcinogenicity in Experimental Animals* – IARC Monographs *Volumes 1-17* (IARC intern. tech. Rep. No. 78/003)

IARC (1979) *Criteria to Select Chemicals for* IARC Monographs (IARC intern. tech. Rep. No. 79/003)

IARC (1982) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Suppl. 4, *Chemicals, Industrial Processes and Industries Associated with Cancer in Humans* (IARC Monographs, Volumes 1 to 29), Lyon, IARC

IARC (1983) *Approaches to Classifying Chemical Carcinogens According to Mechanism of Action* (IARC intern. tech. Rep. No. 83/001)

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Supplement 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, IARC

IARC (1988) *Report of an IARC Working Group to Review the Approaches and Processes Used to Evaluate the Carcinogenicity of Mixtures and Groups of Chemicals* (IARC intern. tech. Rep. No. 88/002)

IARC (1991) *A Consensus Report of an IARC Monographs Working Group on the Use of Mechanisms of Carcinogenesis in Risk Identification* (IARC intern. tech. Rep. No. 91/002)

IARC (2004) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 84, *Some Drinking-water Disinfectants and Contaminants, including Arsenic*, Lyon, IARC, pp. 39–267

IARC (2005) *Report of the Advisory Group to Recommend Updates to the Preamble to the* IARC Monographs (IARC Int. Rep. No. 05/001)

IARC (2006) *Report of the Advisory Group to Review the Amended Preamble to the* IARC Monographs (IARC Int. Rep. No. 06/001)

McGregor, D.B., Rice, J.M. & Venitt, S., eds (1999) *The Use of Short- and Medium-term Tests for Carcinogens and Data on Genetic Effects in Carcinogenic Hazard Evaluation* (IARC Scientific Publications No. 146), Lyon, IARC

Montesano, R., Bartsch, H., Vainio, H., Wilbourn, J. & Yamasaki, H., eds (1986) *Long-term and Short-term Assays for Carcinogenesis—A Critical Appraisal* (IARC Scientific Publications No. 83), Lyon, IARC

OECD (2002) *Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies* (Series on Testing and Assessment No. 35), Paris, OECD

Peto, R., Pike, M.C., Day, N.E., Gray, R.G., Lee, P.N., Parish, S., Peto, J., Richards, S. & Wahrendorf, J. (1980) Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments. In: *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Suppl. 2, *Long-term and Short-term Screening Assays for Carcinogens: A Critical Appraisal*, Lyon, IARC, pp. 311–426

Portier, C.J. & Bailer, A.J. (1989) Testing for increased carcinogenicity using a survival-adjusted quantal response test. *Fundam. appl. Toxicol.*, 12(4), 731–737

Sherman, C.D., Portier, C.J. & Kopp-Schneider, A. (1994) Multistage models of carcinogenesis: an approximation for the size and number distribution of late-stage clones. *Risk Anal.*, 14(6), 1039–1048

Stewart, B.W. & Kleihues, P., eds (2003) *World Cancer Report*, Lyon, IARC

Tomatis, L., Aitio, A., Wilbourn, J. & Shuker, L. (1989) Human carcinogens so far identified. *Jpn. J. Cancer Res.*, 80, 795–807

Toniolo, P., Boffetta, P., Shuker, D.E.G., Rothman, N., Hulka, B. & Pearce, N., eds (1997) *Application of Biomarkers in Cancer Epidemiology* (IARC Scientific Publications No. 142), Lyon, IARC

Vainio, H., Magee, P., McGregor, D. & McMichael, A., eds (1992) *Mechanisms of Carcinogenesis in Risk Identification* (IARC Scientific Publications No. 116), Lyon, IARC

Vainio, H., Wilbourn, J.D., Sasco, A.J., Partensky, C., Gaudin, N., Heseltine, E. & Eragne, I. (1995) [Identification of human carcinogenic risk in IARC Monographs.] *Bull. Cancer*, 82, 339–348 (in French)

Vineis, P., Malats, N., Lang, M., d'Errico, A., Caporaso, N., Cuzick, J. & Boffetta, P., eds (1999) *Metabolic Polymorphisms and Susceptibility to Cancer* (IARC Scientific Publications No. 148), Lyon, IARC

Wilbourn, J., Haroun, L., Heseltine, E., Kaldor, J., Partensky, C. & Vainio, H. (1986) Response of experimental animals to human carcinogens: an analysis based upon the IARC Monographs Programme. *Carcinogenesis*, 7, 1853–1863

004380

3/6/15 Plenary General Remarks:

Group I. Exposure Assessment.

Exposure assessment yes/no.
Few to individual pesticides
Questionaires
Except for the Ag. Health Study.

Used most: glyphosate low production for many
banned: malathion

Group II. Epidemiology

Ag. Heath study. 2 case-control

Midwest Canadian

Exposure Assessments

TCVP - Inadequate for carcinogenity

Parathion - excess risk for melanoma - limited
others inadequate

Malathion - limited

Diazinon - More evidence for cancers
limited NHL, leukemia, lung

Glyphosate - limited NHL
Inadequate MM

Group III - Animal studies

Early - mid 70s Animal bioassay

Limited # of animals

Number of limited ~~factor~~

All studies were considered adequate

FOAs - EPA documents - studies submitted for registration purposes to EPA from Ag. comp.

TCVP - liver tumors mice, Renal carcinoma } sufficient

A switch from limited → sufficient

Group IV.

10 key charac. of agents that cause cancer

TCVP genotox - moderate

004382

Group I

Parathion

Group II

Parathion – Epi. not a lot in humans

Originally: Group III

→ Lung cancer Prostate ← some signals OR 1.5

Group III

Parathion

Sufficient evidence for animal carcinogenicity

mixed adenoma lymphoma

Pals adrenal Mamm. Pancreatic

Group IV

Parathion,

Group I

Malathion – exposure

Group II

Malathion – prostate, NHL

Group III

malathion – mouse liver (M, F) ↑
rat liver
rat mammary

} Sufficient in animals

004383

FOIA - Malathion

MAL / DZN / GLY

→ mechanism operable in humans

Group IV
Malathion Mechanism Upgrade.

Group I
Diazinon

Group II
Diazinon - NHL [Lung cancer] Limited.

Group III
Diazinon 1 study NTP Mouse - liver
Rat - leukemias
[Inadequate evidence in animals]

Group IV

Group I
Glyphosate — detectable in water & food.

Group II
Glyphosate negative NHL
Case-control Glyph. → NHL
AHS negative data.

Group III
Glyphosate - limited to inadequate.

Group IV
Glyphosa

4.3
FOIA data req

005331

From: Ross, Matthew
To: Alavanja, Michael (NIH/NCI) [E]
Subject: Re: Retirement announcement
Date: Thursday, October 1, 2015 6:49:55 PM

Hi Michael,

I just wanted to send along my best wishes to you on the next adventure you are embarking on. I have to admit I was a bit stunned by your email, but trust it will be a rewarding next step.

Indeed, the AHS work had a prominent role at the IARC meeting I attended. The glyphosate issue kind of blew up after we had finished and left. Although it was the rodent cancer bioassays in the case of glyphosate that was really the most controversial issue for glyphosate.

Anyway, I wish you all the best. And I hope our paths may cross again at some future meeting -- it was pleasure working with you.

Best regards,
Matt

On Oct 1, 2015, at 8:44 AM, Alavanja, Michael (NIH/NCI) [E] [redacted] wrote:

Dear friends and colleagues.

I wanted to inform you that I would be retiring from NCI on October 16th. I also wanted to thank you for your contributions to the Agricultural Health Study (AHS) over your many years of service on the AHS Advisory Group. Some of you may even remember, (before we gave our first interview on December 12, 1993) the many obstacles we had to overcome before we received funding, state approvals, and partnership with NIEHS, the USEPA and later NIOSH. Your help was critical.

Judging by the prominent role AHS papers have played in two recent International Agency for Research on Cancer (IARC) monograph meetings in 2015, we can now say the rigorous AHS research is being translated into international public health guidance and policy. The IARC monographs will be available in 2016. Additional IARC monograph meetings on pesticides are planned for the years

ahead. I am sure AHS research will continue to be very influential at these meetings as well.

I believe the best years for AHS research still lie ahead as the cohort ages into the 'cancer prone years'. The NCI work on AHS will now be expertly led by Dr. Laura Bean-Freeman and Dr. Jonathan Hofmann.

I will continue to work on a dozen or so AHS papers while serving as a faculty member at Hood College, in Frederick, MD (a position I also held for the past 25 years).

As of October 17th, my new contact information will be:

[redacted]

My sincere best wishes and gratitude,

Michael

Michael C.R. Alavanja, Dr.P.H.
Senior Investigator,
Division of Cancer Epidemiology and Genetics,
National Cancer Institute,
9609 Medical Center Drive, Rm 6E602
Rockville, Maryland 20892, USA
[redacted]

005035

From: Rusyn, Ivan
To: Kathryn Guyton
Cc: Ross, Matthew; [redacted] LE CURIEUX Frank
Subject: RE: IARC Meeting 112 Reference List for Glyphosate
Date: Friday, February 27, 2015 8:39:56 AM
Attachments: greim_2015_early_online.pdf

Kate,
Thank you. This is an interesting polemical piece. It does not surprise me that when under pressure, the industry can muster a "relevant" publication that goes from submission to acceptance in as little as 7 weeks. Kudos to CRT, a known helper to "informative" publications from the industry stakeholders, for such expediency and relevance.

As I looked through the paper, I believe the most interest in its facts (not conclusions) should be taken by sub-group 3, not group 4. However, I cc here Matt, Matt and Frank so they take a look at small vignettes that are relevant to their sub-sections. There is no other "mechanistic" data in here that warrants attention. I am confident that the IARC monograph will be much more comprehensive and balanced.

Ivan

From: Kathryn Guyton [redacted]
Sent: Friday, February 27, 2015 8:14 AM
To: Rusyn, Ivan
Subject: FW: IARC Meeting 112 Reference List for Glyphosate

Bonjour Professor,
FYI. Do let us know if there are new references you'd like to include from this recent review.
Best,
Kate

From: <FARMER>, "DONNA R [AG/1000]" [redacted]
Date: Friday 27 February 2015 14:25
To: Kate Guyton [redacted]
Subject: RE: IARC Meeting 112 Reference List for Glyphosate

Dear Kate,

I am so sorry the link didn't work.

I have attached the PDF.

Regards,

Donna

005036

From: Kathryn Guyton [redacted]
Sent: Friday, February 27, 2015 4:38 AM
To: FARMER, DONNA R [AG/1000]
Subject: Re: IARC Meeting 112 Reference List for Glyphosate

Dear Donna,

We find the link doesn't work— might you be able to send a PDF?
Thank you,
Best regards,
Kate

Kate Z. Guyton PhD DABT
Responsible Officer, Volume 112
Monographs Section
International Agency for Research on Cancer
150, cours Albert Thomas
69372 Lyon Cedex 08
France
[redacted]

From: <FARMER>, "DONNA R [AG/1000]" <[redacted]
Date: Thursday 26 February 2015 19:14
To: Kate Guyton [redacted]
Subject: RE: IARC Meeting 112 Reference List for Glyphosate

Dear Dr. Guyton,

I wanted to bring to your attention that one of references/publications (Greim et al, 2015) I provided to you that was "in press" and has now be published. This published version has been updated to reflect the revisions in the RAR from the BfR that was posted in January 2015 as discussed below.

Please replace the galley proof with the published version that can be accessed in the link below.

Filename: greim_2015_early_online.pdf (link)

Regards,

Donna

From: FARMER, DONNA R [AG/1000]
Sent: Friday, February 06, 2015 2:34 PM
To: 'Kathryn Guyton' [redacted]
Subject: RE: IARC Meeting 112 Reference List for Glyphosate

005037

Dear Dr. Guyton,

Thank you for your reply.

Yes I did receive your acknowledgement of February 3rd – see our exchange of emails below the one I sent you yesterday.

Regards,

Donna

From: FARMER, DONNA R [AG/1000]
Sent: Thursday, February 05, 2015 3:21 PM
To: 'Kathryn Guyton'; [redacted]
Subject: RE: IARC Meeting 112 Reference List for Glyphosate

Dear Dr. Guyton,

The references in the list I sent you Monday are publicly available however for your convenience I tried to send you a zip file of the copies of the references by IntraLinks Courier™ (a file transfer service). You should have received a separate email with information on how to retrieve the file. As I have not heard from you I assume you have not received this email and therefore not able to access the zip file. As an alternative to providing you copies of those references, this afternoon I have had a Kingston Flash Drive with the zip file sent to you via FedEx International Priority and it should be there typically in two business days.

Also you may or may not be aware that glyphosate is currently undergoing Annex I Renewal, the dossier for this review was submitted in May of 2012 and the draft Renewal Assessment Report (RAR) was made available December 2013. This RAR is publicly available by request on the European Food Standard Authorities (EFSA) web site http://dar.efsa.europa.eu/dar-web/provision.

Germany is the rapporteur Member State (RMS) for this renewal and I would like to bring to your attention that we have just been notified that the Germany Federal Institute for Risk Assessment (BfR) has uploaded a revised RAR to the EFSA Extranet for further consideration in the EFSA Pesticides Peer Review Experts' Meetings. In addition they have also sent the RAR to the European Commission, the Co-RMS Slovakia and the applicant (Glyphosate Task Force).

Included in the reference list I sent you Monday and in the zip file are two extracted sections from the 2013 RAR:

- Germany Federal Institute for Risk Assessment (BfR) Assessment Report

005038

Glyphosate Annex B 6.5.3 Published data on carcinogenicity.
• Germany Federal Institute for Risk Assessment (BfR) Assessment Report Glyphosate Annex B 6.4 Published data on genotoxicity.

When the revised RAR becomes publicly available I will provide any updated information.

Again please don't hesitate to contact me if you have any questions or if I can be of any assistance.

Warmest regards,

Donna

****

Donna R. Farmer, Ph.D.
Product Protection and Nutrition Lead
Toxicology and Nutrition Center
Monsanto Company
800 North Lindbergh Blvd.
Mail Zone O2G
St. Louis, Missouri 63167

[redacted]

From: Kathryn Guyton [redacted]
Sent: Tuesday, February 03, 2015 4:47 AM
To: FARMER, DONNA R [AG/1000]; [redacted]
Subject: Re: IARC Meeting 112 Reference List for Glyphosate

Dear Ms. Farmer,

Many thanks for the information you have sent. We will provide the appropriate scientific articles to the Working Group according to our procedures.

Best regards,
Kate

Kate Z. Guyton PhD DABT
Responsible Officer, Volume 112
Monographs Section
International Agency for Research on Cancer
150, cours Albert Thomas
69372 Lyon Cedex 08
France

005039

[redacted]

From: <FARMER>, "DONNA R [AG/1000]"
[redacted]
Date: Tuesday 3 February 2015 01:48
To: "[redacted]
Subject: IARC Meeting 112 Reference List for Glyphosate

Dear Dr. Guyton,

Please find attached a list of references that Monsanto would like to submit for the Meeting 112 regarding the active ingredient glyphosate.

Please don't hesitate to contact me if you have any questions.

Regards,

Donna

****

Donna R. Farmer, Ph.D.
Product Protection and Nutrition Lead
Toxicology and Nutrition Center
Monsanto Company
800 North Lindbergh Blvd.
Mail Zone O2G
St. Louis, Missouri 63167

[redacted]

005040

005041

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004384

Thoughts on EFSA Response (see NumberedEFSAResponse)

11-13: The CLP classification system is almost identical to the IARC classification system. In these three paragraphs, they are confusing classification with risk assessment. Classification level 1b (ECHA) is almost identical to IARC Classification 2A.

16: The constant use of 6000 pages is misleading; the portion of this document on cancer is much smaller but not easy to quantify because the evaluations are at multiple locations. Maybe as much as 400 pages total.

18: See this article, just published. http://corporateeurope.org/food-and-agriculture/2016/01/eu-review-weedkiller-glyphosate-adds-secrecy-controversy

19: After carefully reading the current RAR, they may be correct in saying that IARC could have used these data; however, second guessing this at this time is wasted effort.

25-29: I have removed most references to BFR in the editorial, sticking mostly with EFSA and RAR. The BFR Addendum is still mentioned becuase of the argument being made in certain parts.

30: Here is the full ECHA Classification Criteria (ECHA 2015)

CATEGORY 1:
Category 1A:
Category 1B:

Known or presumed human carcinogens
A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:
Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or
Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.
The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from:
– _human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or
– _animal experiments for which there is sufficient [1] evidence to demonstrate

	animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.
CATEGORY 2:	Suspected human carcinogens The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited(1) evidence of carcinogenicity in human studies or from limited

37, 43-44: Their interpretation of the meta-analysis is contradictory to their argument. It suggests a very limited understanding of the issues involved.

39: There is no category of "very limited" in their guidance documents. From the ECHA (2015) guidance, does this look familiar?

Carcinogenicity in humans
The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:
– sufficient evidence of carcinogenicity: a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence;
– limited evidence of carcinogenicity: a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.

46: This is better addressed in the Editorial.

49-50, 61: I searched all of the documents for "historical" to see if I could

understand what they are referring to. In several cases in the text added by the EFSA Review, they mention obtaining historical controls from the same laboratory, but provide absolutely no details. For example "Although the increase in lymphoma incidence in the study by (XXXXX 2001, [25]) was statistically significant in both sexes, it was still within the (small) historical control range of the performing laboratory for females. No evidence of a similar effect in female mice was obtained in any other study." The only detailed historical control evaluation is the BfR Addendum. I have altered the Editorial text to reflect this.

52-53: While I would argue that this is true in epidemiological studies, I firmly disagree with this argument for the animal studies, for the obvious reasons. If you do a study where you control everything to be the same except dose, and you use multiple doses, you are looking for a pattern with respect to those doses. Hence, a dose-response evaluation, like a trend test, is most appropriate and more powerful. In addition, they discard effects at multiple points in the document because the effect was only seen at low doses. The logic here is silly.

57: Hmmm, evidence of renal tumors in three mouse studies, hemangiosarcoma in two mouse studies and malignant lymphoma in two mouse studies out of five studies is not consistent evidence? In addition, if I have inconsistent results, say one positive and one negative study, why do I presume the negative finding is the correct interpretation?

58-59: I can find no reports of hyperplasia of any kind in kidney. It is not clear to me why the findings in the liver, bladder, etc. support this statement?

65: 1997 was positive for trend. 2009 was an 18 month study with a 5-fold lower dose. 1993 is in an unknown substrain, 24 months at a 4-fold lower dose.

66: It is hard to see where this infection issue is coming from. The RAR says this about this study:

The high background incidence of malignant lymphoma in Swiss mice was confirmed in a literature search that was performed by the RMS on request of the Pesticides Peer Review 125 expert meeting. Its results are given in detail in Vol. 3 (B.6.5.2). According to older articles, control incidences in male mice of Swiss or Swiss-derived strains may reach 18–27.5 % and exceed 36 % in females (Sher, 1974, Z22020; Roe and Tucker, 1974, ASB2015-2534; Tucker, 1979, Z83266). Even though these historical rates were still lower than what was seen in the study by (2001, ASB2012-11491) at least at the higher dose levels, they provide clear proof that Swiss mice are prone to developing lymphoreticular tumours. In a more recent publication, Tadesse-Heath et al. (2000, ASB2015-2535) even mentioned a nearly 50% lymphoma (mostly of B cell orgin) incidence in a colony of CFW Swiss mice. The latter authors emphasised the contribution of widespread infections with murine oncogenic viruses to the high but remarkably variable incidence of tumours of the lymphoreticular system. No information is available on possible abundance of

such viruses in the mouse colonies from which the animals used in the glyphosate studies were obtained.

I have extracted the relevant 10 pages from the RAR and included them here (Swiss Mouse Study.pdf). The actual study (better formatted) is in the RAR pages 1013 to 1023 in your PDF Viewer (not as numbered by the EFSA). Anyway, it appears to me that they speculate about this, but there is no indication of such an infection in these animals in this study. They even say toward the end "It is not known to which extent such a latent infection might have contributed to lymphoma incidences reported earlier or even in the studies described in this RAR."

The EFSA Peer_Review document says "The study was re-considered during the second experts' teleconference (TC 117) as not acceptable due to viral infections that could influence survival as well as tumour incidence – especially lymphomas." I can find no description of this meeting or the evidence.

efsa
European Food Safety Authority

EXECUTIVE DIRECTOR

13 January 2016
Ref. BU/JK/JR/aa (2016) – out-15124233

Prof. Christopher J. Portier
Senior Contributing Scientist
Environmental Defense Fund
1875 Connecticut Ave NW, Ste 600
Washington, DC 20009
United States of America

Subject: Open letter: Review of the Carcinogenicity of Glyphosate by EFSA and BfR

Dear Professor Portier,

(1) First of all, I would like to thank you for sight of the open letter dated 27 November 2015 which you sent to the EU Commissioner for Health and Food Safety Vytenis Andriukaitis regarding EFSA's recent re-assessment of glyphosate. I am writing directly to you and to the co-signatories of your letter, with whom I trust you will share my response.

(2) I would first like to address some of the general points you raise, particularly regarding the regulatory process for the peer review of pesticides in the European Union and the transparency of that process.

(3) Enclosed is also an Annex that gives detailed answers to the scientific questions you raised in your letter. These include, for example, explanations on the evidence from animal carcinogenicity studies, EFSA's interpretation of the tumours reported in the IARC monograph, and mechanistic information.

(4) I would like to make one over-riding point. Glyphosate is currently a keenly debated issue, which makes it especially incumbent on those of us involved in its evaluation to describe clearly the legal frameworks in which we work. In that way, we avoid confusing the policy makers who rely on our advice and the general public who depend on us to maintain the highest standards in protecting public health.

IARC assessment as a possible first step in a full assessment

(5) As the WHO states on its website in the Preamble to the IARC Monographs, IARC evaluations can represent a first step in carcinogen risk assessment to be considered – if available – by national and international authorities such as EFSA when carrying out their own assessments.

(6) I agree that IARC carries out an important role in the screening assessment of the carcinogenic potential of agents. However, we should not compare this first screening assessment with the more comprehensive hazard assessment done by authorities such as EFSA, which are designed to support the regulatory process for pesticides in close cooperation with the Member States in the EU.

Glyphosate is not the first chemical where there has been a difference between the IARC screening and the final comprehensive assessment by regulatory bodies. If you compare IARC categorisations with the EU harmonised classifications, you will find substances with equivalent classifications and others with different classifications. This shows that although the IARC screening has been considered, it has not always been confirmed.

EFSA's assessment of glyphosate is an essential part of the EU regulatory system in relation to pesticides - widely regarded as one of the strictest in the world. This system was most recently updated in 2009 through co-legislation agreed by the European Parliament and the Member State governments acting within the Council of the European Union (EU Regulation 1107/2009).

This is the system EFSA has followed in the assessment of hundreds of active substances since 2003. These assessments have identified potential concerns for human health and the environment and allowed the European Commission and Member States to establish requirements for the safe use of pesticides in Europe. They have also led to the removal from the EU market of more than 40 active substances and their corresponding formulations. It is the same system that was used to assess the risk to bees from neonicotinoids, which were latterly subject to an EU moratorium.

EFSA's assessment was the first published after the release of the IARC monograph in July and other organisations worldwide are conducting similar assessments, including the Joint FAO/WHO Meeting on Pesticide Residue, which is scheduled to publish its own assessment of glyphosate in May 2016 and has asked EFSA for all available scientific information from its own recent assessment to allow it to do this.

Different classification systems

EFSA uses a classification system developed specifically for chemicals by the United Nations (UN-GHS for classification and labelling of chemicals). The EU was one of the first jurisdictions in the world to implement this system, which allows for the identification of the hazards of each chemical and mixtures (e.g. pesticides formulations)

The screening aim of the IARC classification scheme explains why chemicals in pesticides such as glyphosate, or red meat, or frying food at high temperatures, can be included in the same IARC category as being *probably carcinogenic*. But it is important to remember that these classifications are only one part of the body of information in a risk assessment and on which public health decisions may be based.

IARC's broad screening covered both the active substance glyphosate and glyphosate-based pesticide formulations, whereas EFSA focused only on the active substance as it is required to do by EU legislation. In the EU, individual Member States are responsible for evaluating the safety of pesticide formulations used on their territory, including the assessment of the other ingredients (the co-formulants).

EFSA invites IARC to discuss scientific divergences

In an effort to clarify scientific divergences, and in line with EFSA's principles of openness and transparency, EFSA and IARC have agreed to meet early in 2016 to discuss the different evidence and the different methodologies that the two organisations have used. Both of these elements play a role in explaining the divergences between the IARC and EFSA assessments of the carcinogenic potential of glyphosate and we look forward to exchanging views with IARC along these lines.

EFSA carried out open and transparent assessment

15 Finally, I would like to address the issue of transparency. I strongly disagree with your contention that EFSA has not applied open and objective criteria to its assessment. EFSA implemented the legal requirement to carry out a scientific peer review with Member States, alongside expert and public consultations, in a transparent manner, as it does with all pesticide active substances.

16 The EFSA Conclusion and all related background documents which run to around 6,000 pages have been published on EFSA's website[1]. These documents include the public consultation report showing how all comments were addressed, both from Member States and from the 29 submissions which came from individuals and organisations, including a number of environmental NGOs.

17 An essential element of any regulatory scientific assessment is to ensure consistency across evaluations. The views of Member State experts, who may collect input from several public organisations within their Member State before submitting consolidated comments, are discussed in expert groups covering different scientific areas, such as ecotoxicology or mammalian toxicology. Experts from IARC, the JMPR, ECHA and US EPA were invited as observers to the expert consultations to discuss the carcinogenicity of glyphosate. Reports of these meetings or teleconferences are also published in the background documents on EFSA's website.

18 Additionally, for the sake of transparency, EFSA invites the Member State scientists who take part in the peer review to submit a Declaration of Interest (DoI), although they are not obliged in the legislation to do so. These DoIs are published on EFSA's website. The Member State scientists are affiliated to a broad range of public institutions across the EU.

19 I wish to make a final but important point regarding transparency. The background documents display detailed information on how EFSA and Member States appraised each study, including industry sponsored studies, and how all those which participated, except Sweden, concluded that glyphosate is unlikely to pose a carcinogenic hazard to humans.

20 The type and amount of information published by EFSA about these studies is comparable to that found in the US EPA and JMPR reports used by IARC for the assessment of carcinogenicity in animals. It is also comparable to the type and amount of information provided in papers in the open scientific literature. IARC, and any interested parties, are welcome to review the information EFSA has published on its website.

21 In conclusion, I hope very much that this letter goes some way to clarifying any doubts you may have had about the process which EFSA has followed in its assessment of glyphosate or about our commitment to ensuring that this process is as open and transparent as possible.

22 Additionally, I also trust the scientific detail you find in the attached Annex will help to further your understanding of the approaches and methods we used in reaching our conclusions.

Yours sincerely,

Bernhard Url

[1] http://www.efsa.europa.eu/en/press/news/151119a

Annex: Specific responses to the open letter sent by Prof. Christopher Portier and others to Vytenis Andriukaitis, EU Commissioner for Health and Food Safety

cc (email only):

Dr. Vytenis Andriukaitis, European Commissioner for Health and Food Safety

Mr. Phil Hogan, European Commissioner for Agriculture and Human Development

Mr. Xavier Prats Monné, Director-General, European Commission DG Health and Food Safety

Dr. Ladislav Miko, Deputy Director-General, European Commission DG Health and Food Safety

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Dr. Giovanni La Via, Chair, ENVI Committee of the European Parliament

Mr. Christian Schmidt, German Federal Minister of Food and Agriculture

Dr. Helmut Tschiersky, President, BvL

Professor Dr. Dr. Andreas Hensel, President, BfR

Dr. Christopher Wild, Director, IARC

Mr. Jim Jones, Assistant Administrator, USEPA

European Food Safety Authority

EXECUTIVE DIRECTOR

ANNEX

Specific responses to the open letter sent by Prof. Christopher Portier and others to Vytenis Andriukaitis, EU Commissioner for Health and Food Safety

This annex addresses specific scientific comments made in the open letter of 27 November 2015 to Commissioner Andriukaitis on a review of the carcinogenicity of glyphosate by EFSA and the BfR, signed by Prof. Christopher Portier and 95 scientists (hereafter referred to as the 'open letter'). The annex responds also to direct quotes from the open letter.

I. General comment

The open letter states: "Addendum 1 (the BfR Addendum) of the RAR[2] discusses the scientific rationale for differing from the IARC WG conclusion."

It is noted that the open letter does not always refer correctly to a) the German Rapporteur Member State (RMS) assessment and proposal; b) the outcome of the experts' discussions; and c) the final conclusion by EFSA (EFSA, 2015a).

The revised Renewal Assessment Report (Germany, 2015) presents the final views of the Rapporteur Member State (Germany), taking into account the comments received from the public consultation and the discussions held with the other EU Member States and EFSA. It includes the Addendum assessing the findings of the IARC monograph.

The Peer Review Report (EFSA, 2015b) captures transparently all comments received on the draft Renewal Assessment Report (Germany, 2013) and follow-up submissions thereof, including Addendum 1, the report from the discussions at the various expert meetings, the comments on the additional information requested by EFSA and the comments submitted on the draft EFSA Conclusion and how these have been addressed.

The two documents mentioned above support EFSA's final view, presented in the EFSA Conclusion (EFSA, 2015a). EFSA has also published a complementary paper summarising its assessment of the genotoxicity and carcinogenicity assessments, which is also available on the EFSA website (EFSA, 2015c).

EFSA notes that the EU assessment on the potential carcinogenicity hazard of glyphosate is based on the UN Global Harmonised System of classification and labelling of chemicals (United Nations, 2003 and posterior revisions every two

years), implemented in the EU through the Classification, Labelling and Packaging (CLP) Regulation[1]. The hazard categories are:

- Category 1: Known or presumed human carcinogens
  - Cat 1A: Known to have carcinogenic potential for humans (human data)
  - Cat 1B: Presumed to have carcinogenic potential for humans (animal data)
- Category 2: Suspected human carcinogens
- No classification: classification criteria not met

31 IARC uses a different classification scheme, with different groups[2]; however, "there is a strong link between IARC and CLP classification criteria" (ECHA Guidance on the Application of the CLP Criteria 2013, 2015), as the definitions for sufficient and limited evidence as defined by IARC are part of the CLP criteria.

II. Evidence from human epidemiological studies

a) Overall considerations on scientific evidence from epidemiological studies

32 The open letter states: "The EFSA conclusion that 'glyphosate is unlikely to pose a carcinogenic hazard to humans' is inappropriate when available data support the determination of limited evidence of carcinogenicity in humans."

33 According to the Guidance on the Application of CLP criteria (ECHA 2013, 2015): "*The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:*

> *— sufficient evidence of carcinogenicity: a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence;*
> *— limited evidence of carcinogenicity: a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence"*

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[1] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, 1-1355.

[2] IARC classification for carcinogenic agents (not just chemicals)
- Group 1. The agent is carcinogenic to humans
- Group 2.
  - Group 2A. The agent is probably carcinogenic to humans
  - Group 2B. The agent is possibly carcinogenic to humans
- Group 3. The agent is not classifiable as to its carcinogenicity to humans
- Group 4. The agent is probably not carcinogenic to humans

34 With regard to the criteria for the definition of "sufficient" and "limited" evidence, IARC acknowledges the possibility of deviating from the indications based on experts' judgement, as all relevant scientific data may be assigned with a higher or lower category than a strict interpretation of the criteria (as referred to in the IARC preamble 2006).

35 Regarding epidemiological studies, the IARC and EFSA assessments are based on the same evidence.

36 In line with the CLP criteria and ECHA guidance (ECHA, 2013; 2015), the two key points considered in the EU assessment are:

- The assessment of chance, bias or confounding effects in the statistical associations.
- The credibility of the causal interpretation. In this sense, it should be noted that the different conclusions regarding genotoxicity and carcinogenicity in animals from IARC and EFSA lead to different views regarding the credibility of the causal interpretation.

37 In the IARC Non-Hodgkin Lymphoma (NHL) meta-analysis, Schinasi and Leon (2014) reported on the relationship between 14 groups of herbicides and insecticides. In nine (64%) of the groups they found either the group as a whole, or one or more of the individual pesticides within those groups, to be statistically significantly associated with risk for NHL.

38 Considering the above CLP criteria and, in particular, "the assessment of chance, bias or confounding effects in the statistical associations", the question needs to be addressed as to whether these statistical relationships are supportive of a causal relationship between exposure and the specific active ingredients in these pesticides. As discussed in the epidemiological literature, specific concerns in this regard include:

- characterisation and assessment of the risk factor of interest, i.e. in this case the active ingredient glyphosate itself;
- variation in disease definition;
- characterisation and measurement of exposure to the risk factor;
- confounding by other risk factors – including other pesticides; and
- exploratory statistical analyses, without correction for multiple testing.

39 In contrast to the IARC evaluation of the epidemiological studies as being of limited evidence, the EU experts have concluded that the human evidence is very limited and, therefore, insufficient for classification under the CLP criteria. There is a minority view (one EU Member State) considering that the information is sufficient for limited evidence in humans according to the CLP Regulation (Category 2); this minority view can be considered in line with the IARC assessment of epidemiological studies as limited evidence. This conclusion and the minority opinion are both reported in the Conclusion (EFSA, 2015a) and the details are presented in the Peer Review Report (EFSA, 2015b).

b) Specific considerations on scientific evidence from epidemiological studies

40 The open letter states: "To provide a reasonable interpretation of the findings, an evaluation needs to properly weigh studies according to their quality rather than simply count the number of positives and negatives. The meta-analyses cited in the IARC monograph and done by WG are excellent examples of an objective evaluation of the existence positive association; both meta-analyses showed a statistically significant association."

41 EFSA notes that, in reality, the meta-analyses that are mentioned weigh the studies based on the confidence limits of the Odds Ratio, which is based on its standard error, which in turn depends on the study size. Thus the weighing does consider the number of cases/subjects at least indirectly. Furthermore, among the studies included in this meta-analysis, there was no other stated weight-adjustment for study design or elements of study quality.

42 The open letter states: "There were only 92 NHL cases included in the AHS [Agricultural Health Study] unadjusted analysis and fewer in the adjusted analyses, compared to 650 in a pooled case-control analysis from the Unites States."

43 EFSA notes that a comparison is made between the relative strength of the De Roos *et al.* (2003) case-control study versus the De Roos *et al.* (2005) cohort study, by using just one figure from each of these two studies. This is misleading. EFSA suggests that the following numbers from the two studies should be considered instead.

De Roos *et al.* (2003) case control study (analyses of pooled data from three studies)

	Cases	Controls	Total
Exposed	36	61	97
Non-exposed	614	1,872	2,486
	650	1,933	2,583

De Roos *et al.* (2005) cohort study

	NHL	No NHL	Total
Exposed	71	40,964	41,035
Non-exposed	21	13,259	13,280
	92	54,223	54,315

44 Taking this full set into account, it is not clear why the power of the De Roos *et al.* (2005) study would be in doubt, when comparing it to its predecessor case-control study (De Roos *et al.*, 2003). In fact, please note that even the IARC meta-analysis (Schinasi and Leon, 2014) gives a (somewhat) higher weight to the De Roos *et al.* (2005) study (21%) than to the De Roos *et al.* (2003) study (15%).

c) Conclusions

As highlighted by Nordström *et al.* (1998), and in contrast to other occupational exposures, farming can involve exposure to many chemicals. This is one reason why the question as to whether human exposure to glyphosate formulations, let alone glyphosate by itself, lead to NHL is difficult to answer through epidemiological studies. One approach to dealing with such an issue is to assess an entire class of compounds, without determining which specific chemical(s) might be responsible. For pesticides the approach is to examine each pesticide active substance independently, as is being done for these and other regulated substances in various jurisdictions worldwide.

III. Evidence from animal carcinogenicity studies

a) General comments

In the open letter it is assumed that the use of historical control data was the only reason in the EFSA assessment for considering that the studies indicating non-statistically significant differences in the pair-wise analysis but significant trends were insufficient for supporting classification under the CLP Regulation.

This is not correct, as the EFSA assessment (EFSA, 2015a) is based on weight of evidence, fully in line with the CLP criteria and the ECHA guidance (ECHA, 2013; 2015), regarding the biological relevance of observed incidences for the assessment of the carcinogenicity potential of glyphosate:

"No evidence of carcinogenicity was confirmed by the large majority of the experts (with the exception of one minority view) in either rats or mice due to a lack of statistical significance in pair-wise comparison tests, lack of consistency in multiple animal studies and slightly increased incidences only at dose levels at or above the limit dose/MTD, lack of preneoplastic lesions and/or being within historical control range. The statistical significance found in trend analysis (but not in pair-wise comparison) per se was balanced against the former considerations." (EFSA, 2015a)

In addition, the open letter claims that the historical control data were not considered properly, but as explained below this is not correct either.

The scientific principles used by EFSA in the evaluation of animal carcinogenicity studies, in line with the regulatory context of our evaluation, are summarised below; the details are included in the background documents supporting the EFSA conclusion (Germany 2015; EFSA 2015b).

EFSA and the experts of the member countries, including the RMS, had access to and evaluated the original studies. Comprehensive description and evaluation of the new long-term studies by the RMS in its Renewal Assessment Report was not taken into consideration by IARC even though this information was publicly available from April 2014. IARC used a new interpretation and statistical evaluation (by trend

tests) of tumour incidences that are from older studies and have been discussed by the JMPR and the US-EPA.

b) Statistical assessment

EFSA is of the opinion that the planning of a study before the initiation of the experimentation as established in the respective protocol – which includes the planned statistical analysis – is a key element in assessing the quality of a study; therefore deviations from the statistical analysis used by the study authors should be limited and properly justified. This is in line with OECD recommendations: "*The central concept of this document is that the experimental design represents the strategy for answering the question of interest and that the specific statistical analyses are tactical methods used to help answer the questions. Therefore, the statistical methods most appropriate for the analysis of the data collected should be established at the time of designing the experiment and before the study starts.*" (OECD, 2012).

The studies under consideration were designed for pair-wise comparisons, and this was the statistical method considered in the EU assessment. IARC based its assessment on previous evaluations of studies as carried out by the US-EPA and the FAO/WHO JMPR, which included a Cochran analysis. In 2014 the US-EPA decided to disregard the result of the analysis because the biological relevance of the findings could not be proven.

As indicated in the open letter, in some studies the same data are statistically significant or not, depending on the selected statistical method. It should also be noted that there are no valid studies with statistically significant effects confirmed by both statistical approaches. Based on these results, the biological relevance of the results (see below) was balanced against the inconsistency observed in the statistical results.

c) Assessment of biological relevance

As indicated before, the EFSA conclusion regarding carcinogenicity in animals considered the different statistical assessments (significant trends but non-significant effects in the pair-wise comparison with the concurrent control group) and conducted a scientific assessment of the biological relevance of the observed tumour incidences.

As mentioned in the EFSA Conclusion (EFSA, 2015a), the EU assessment is based on weight of evidence, in line with the CLP criteria and ECHA guidance (ECHA, 2013; 2015), focusing on four main arguments:

- Lack of consistency in multiple animal studies. The CLP criteria (Section 1.1.1.) require that: "*The quality and consistency of the data shall be given appropriate weight*" and that: "*Both positive and negative results shall be assembled together in a single weight of evidence determination.*" Based on the evidence available for the EU assessment, which included five additional valid long-term toxicity-carcinogenicity studies known of but not assessed by

IARC, inconsistent effects were observed in the tumour incidences both within (lack of dose response) and between studies (inconsistency between results observed at the same dose in different equivalent studies). Some trends were observed only in one sex. On this point the ECHA guidance (ECHA, 2013; 2015) considers that: *"If tumours are seen only in one sex of an animal species, the mode of action should be carefully evaluated to see if the response is consistent with the postulated mode of action."* However, no assessment of a sex related mechanism is provided in the IARC assessment.

- Incidences only at dose levels at or above the limit dose/maximum tolerated dose (MTD). The IARC monograph reports for several studies significant body weight reductions at the highest doses, which are in fact the doses triggering the statistical significance of the trend analysis. No further assessment of the possibility of a confounding effect of excessive toxicity at these test doses is reported in the monograph. Excessive toxicity – for instance, toxicity at doses exceeding the MTD – can affect the carcinogenic responses in bioassays. Such toxicity can cause effects such as cell death (necrosis) with associated regenerative hyperplasia, which in turn can lead to tumour development as a secondary consequence, unrelated to the intrinsic potential of the substance itself to cause tumours at lower and less toxic doses (ECHA, 2013; 2015).

58

In line with the CLP and UN-GHS criteria, ECHA has provided clear guidance on this aspect of the assessment: *"If a test compound is only found to be carcinogenic at the highest dose(s) used in a lifetime bioassay, and the characteristics associated with doses exceeding the MTD as outlined above are present, this could be an indication of a confounding effect of excessive toxicity. This may support a classification of the test compound in Category 2 or no classification."* In addition, it is clear that the trend analysis should not be used for studies where high tumour incidences are observed only at doses exceeding the MTD; and the statistical assessment should focus on the pair-wise comparison with the concurrent controls, which did not show statistically significant differences for any of the valid studies on glyphosate. In addition to the significant body weight loss reported in the IARC monograph, other signs of excessive toxicity reported at high doses included hepatic centrilobular hypertrophy, bladder epithelial hyperplasia, ulcerations, etc.

59

- Lack of preneoplastic lesions in organs where tumours occurred, as indicated in the histological evaluations of several studies, which failed to show a histopathological continuum possibly indicating an evolution to frank neoplasms.

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- Incidences being within historical control range. EFSA notes that, of the four key elements used by EFSA, this is the only one mentioned in the open letter. It is also noted that the open letter incorrectly reports how historical control data are used in the EFSA assessment. First, the open letter includes the following reference to the IARC preamble: *"It is generally not appropriate to discount a tumour response that is significantly increased*

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compared with concurrent controls by arguing that it falls within the range of historical controls." However, it should be noted that all incidences reported from reliable studies were not statistically significant when compared to the concurrent controls in the pair-wise comparisons. Second, it seems that the letter signatories have misinterpreted the efforts made by the German RMS to get supportive information for those studies with no valid historical controls. The Peer Review Report (EFSA, 2015b) confirms that EFSA conducted a specific check regarding the use of historical control data, requested additional information during the clock-stop procedure and only considered valid the historical control data from the performing laboratory in line with the international recommendations (e.g. ECHA, 2013; 2015).

d) Additional considerations of the tumours reported in the IARC monograph

62 For the assessment of tumours in mice, IARC and EFSA considered two and five studies, respectively.

Renal tumours reported in mice

63 The open letter mentions *inter alia a* significant positive trend for renal tumours in CD-1 mice.

In a 1983 study, a marginally increased incidence of renal tumours was reported in male Charles River CD-1 mice, not statistically significant in a pair-wise comparison after adjusting for higher survival in the high dose group; no renal tumour was observed in females. The renal tumours could not be linked to glyphosate administration due to several considerations: the trend analysis reported by IARC does not take into account the higher survival rate at the high dose and the fact that no preneoplastic lesions were observed and therefore a morphological continuum could not be established. Additionally, concomitant general toxicity was observed at the high dose level (4,841 mg/kg bw per day) – such as reduced body weight, histopathological changes in the bladder and liver – that could be responsible for the occurrence of tumours and not a direct effect of the test substance. It is therefore concluded that the reported incidence of renal tumours is most likely a chance finding, not related to glyphosate administration.

64

Three more recent studies (1993, 1997 and 2009) performed on CD-1 mice did not show dose-related increased incidences of renal tumours. In the 1993 study, renal tubular adenoma and carcinoma cases were observed in the control and low-dose groups only. In the 1997 study, no renal carcinomas were observed, and two adenomas occurred only at a very high dose (exceeding 4,000 mg/kg bw per day). No renal tumour or other renal lesions were observed in the 2009 study in any group.

65

A fifth study performed on Swiss albino mice (2001) was concluded to be unreliable since the health of the animals in the study was clearly compromised due to viral infections in all groups including concurrent control.

66

67 In conclusion, the evidence from four valid studies using CD-1 mice does not indicate that the observed incidences of renal tumours are test substance-related. This was also the conclusion in the EPA publication (US-EPA, 1986), which was analysed by IARC.

Haemangiosarcomas reported in mice

68 With regards to haemangiosarcomas, for which statistically significant trends by Cochran-Armitage test but not by pair-wise comparisons could be observed in two out of four valid studies at the highest dose tested, both incidences observed were within the performing laboratory's historical control data and therefore concluded not to be linked to glyphosate administration.

Malignant lymphomas reported in mice

69 Increased trends of malignant lymphomas, one of the most common spontaneously occurring neoplasms in mice, were observed in male mice in three (1997, 2001 and 2009) of the five studies. Females presented in general higher incidences than males but statistical significance was not achieved and dose-response was not evident. In one study (1997), there was a positive trend test but the incidences remained clearly within the performing laboratory historical control data. A second study using lower dose levels, and for which no reliable laboratory historical control data were available, also showed a positive trend (2009). However, for both studies pairwise comparisons did not reveal a statistically significant increase. The third study (2001) was concluded to be unreliable for the reasons expressed above (occurrence of viral infection). Two additional studies (1983 and 1993) neither showed a positive trend nor revealed a significant increase in tumour incidences in pair-wise comparison. Using a weight of evidence approach by also considering the known high background incidence of this tumour type in mice, it was concluded that these tumours are spontaneous in origin and not test substance-related.

70 For the assessment of tumours in rats, IARC and EFSA considered six and nine studies, respectively.

Pancreatic islet cells in rats

71 Regarding rat studies, from nine studies submitted, seven did not present any increased incidence of neoplastic lesions that could be related to glyphosate administration. Nevertheless, IARC reported significant positive trends in two studies. In one study from 1981, a statistically significant (according to a pair-wise comparison) increased incidence of islet cells adenomas was limited to the low dose level; in the absence of a dose-response relationship, the finding cannot be linked to glyphosate administration. Similarly, in a 1990 study using much higher dose levels, a significant increase over the control incidence was observed only for the low dose group. There was no progression to carcinoma. Thus, no dose-response relationship could be established with regards to the incidence of pancreatic islet cells adenomas and no confirmation was obtained in any of the other long-term studies in rats.

Hepatocellular and thyroid C-cell adenomas in rats

72 Regarding positive trends reported by IARC for hepatocellular adenomas in males and for C-cell adenomas in females, the lack of statistical significance in a pair-wise

comparison, the comparable incidence observed in the opposite sex and the lack of consistency of the finding in the many other studies (eight studies) led to the conclusion that the neoplastic findings are unlikely to be test substance-related.

e) Conclusion

The arguments expressed in the open letter reflect a misunderstanding of the evidence used for the EFSA evaluation. The biological relevance of each study and the overall evidence on animal carcinogenicity was properly assessed during the EFSA evaluation. In contrast, the IARC assessment focused on finding statistically significant "trends" in specific studies, but presented no information on how it considered the biological relevance and in particular the inconsistencies and effects only observed at doses at or exceeding the MTD, even when it is clear that the trend was significant only due to the incidences observed at the highest dose at which significant weight reduction and other indications of excessive toxicity had been observed. In fact the statistical trend, without assessing the biological relevance of the results, seems to be the only justification in the IARC monograph for deviating from the previous evaluation of the same animal studies by the WHO/FAO JMPR expert group, which concluded that glyphosate does not have carcinogenic potential (JMPR, 2004).

IV. Mechanistic information

a) Genotoxicity

No scientific elements are presented in the open letter and the allegations focus on procedural issues. The first allegation related to genotoxicity is that BfR's use of unpublished evidence makes it impossible for any scientist not associated with the BfR to review its conclusions. This is not the case: EFSA and the BfR's appraisal of the studies you refer to is available in the EFSA Conclusion and supporting documents (published on our website) with a level of detail at least comparable to the US-EPA and WHO/JMPR reports relied on in the IARC monograph. The studies are made publicly available for scientific scrutiny and were available at the time you wrote your letter.

Regarding the weight given to the different studies, as the EFSA assessment focuses on the active substance glyphosate and the assessment of genotoxicity in humans, *in vivo* mammalian studies conducted with the active substance were considered more relevant, particularly when the technical specifications and impurity profile of the tested substance were reported. According to the IARC monograph, the studies with exposed humans were conducted with formulated products, not with the active substance, and there is no indication in the monograph of any attempt to establish the possible role of the co-formulants, even when other studies (*in vitro* or in animals) report negative effects for the active substance and positive effects for the formulated products.

Sixteen *in vivo* studies in somatic cells and two *in vivo* studies on germ cells were reported on rodents treated orally with dose levels of up to 5,000 mg/kg bw or via

intraperitoneal injections. All studies conducted according to internationally validated guidelines and some non-GLP published studies gave negative results, while two non-GLP studies were positive in mice treated intraperitoneally with dose levels in the range of the intraperitoneal LD_{50} for mice, one study presenting major flaws. Conflicting results were obtained regarding DNA adduct formation; induction of DNA strand breaks was observed in mice treated intraperitoneally with doses close to or in excess of the LD_{50}.This induction may be caused by secondary effects of cytotoxicity. No genotoxic effects on germ cells have been detected in rats or mice treated orally at dose levels up to 2,000 mg/kg bw.

b) Oxidative stress and use of scientific literature

77 The available studies and reports on the oxidative stress potential of glyphosate, and its causal link, if any, to the occurrence of tumours, are extremely limited. The possibility that glyphosate could cause oxidative stress was indeed discussed during the EFSA peer review: oxidative stress was recorded only in one study in rats administered with pure glyphosate, in combination with cytoxicity and degenerative effects in the targeted organ. Thus, in consideration of the extremely limited database and because of the lack of evidence for carcinogenic potential of glyphosate, no further consideration regarding the mode of action was necessary.

78 EFSA agrees with the statement in the open letter regarding the relevance of scientific literature, e.g. for understanding the mechanism of action. The EU regulatory system requires an assessment of scientific peer-review data published in the previous 10 years to be presented in the dossier, and EFSA has developed a guidance document for ensuring a proper implementation of this requirement (EFSA, 2011); in addition, the regulation allows the submission of additional data to the RMS; additional data can also be submitted during the public consultation. Scientific peer-reviewed publications support several recommendations in the EFSA conclusion, such as the proposal for considering specifically the genotoxicity of the formulated products during the MS evaluations.

c) Conclusion

79 Considering a weight of evidence approach, taking into account the quality and reliability of all available data, it is concluded that glyphosate is unlikely to be genotoxic *in vivo* and does not require hazard classification regarding mutagenicity according to the CLP Regulation. It is noted that unpublished studies that were the core basis of the EFSA evaluation were not available to the IARC experts as reported in the IARC monograph 112 on glyphosate.

V. Active substance versus formulations

80 In the summary of the open letter a distinction is made between the assessment of the active substance and the assessment of the formulations. "*The most parsimonious scientific explanation of the cancers seen in humans and laboratory*

animals supported by the mechanistic data is that glyphosate is a probable human carcinogen. On the basis of this conclusion and in the absence of contrary evidence, it is reasonable to conclude that glyphosate formulations should also be considered probable human carcinogens." IARC did not try to differentiate whether the effects were linked to the active substance, other ingredients (co-formulants), or combined effects of several ingredients, even when the evidence suggested negative effects for glyphosate and positive effects for a formulated product. The IARC monograph states that formulated products contain other ingredients, and mentions specifically polyethoxylated tallowamine, a co-formulant considered of potential concern and recently assessed by EFSA (EFSA, 2015d).

VI. Summary

81

EFSA considers that the arguments brought forward in the open letter do not have an impact on the EFSA conclusion on glyphosate. The arguments expressed in the open letter reflect a misunderstanding of the evidence used for the EFSA evaluation.

82

As reported in the EFSA Conclusion (EFSA, 2015a), there is very limited evidence for an association between glyphosate-based formulations and non-Hodgkin lymphoma, and overall evidence is inconclusive for a causal or otherwise convincing associative relationship between glyphosate and cancer in human studies. There is no evidence of carcinogenicity in either rats or mice due to a lack of statistical significance in pair-wise comparison tests, lack of consistency in multiple animal studies and slightly increased incidences only at dose levels at or above the limit dose/MTD, lack of pre-neoplastic lesions and/or being within historical control range. The statistical significance found in trend analysis (but not in pair-wise comparison) per se was balanced against the former considerations. Considering a weight of evidence approach, taking into account the quality and reliability of all available data, it is concluded that glyphosate is unlikely to be genotoxic in vivo and does not require hazard classification regarding mutagenicity according to the CLP Regulation.

VII. References[3]

De Roos *et al.*, 2003. De Roos A. J., Zahm S. H., Cantor K. P., Weisenburger D. D., Holmes F. F., Burmeister L. F., Blair A., 2003. Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. Occupational and Environmental Medicine vol.60, 9 (2003)

De Roos *et al.*, 2005. De Roos A. J., Blair A., Rusiecki J. A., *et al.*, 2005. Cancer incidence among glyphosate-exposed pesticide applicators in the agricultural health study, page 49-54. Environmental Health Perspectives, VOLUME 113, NUMBER 1

[3] An updated list of studies relied upon for the EU peer review process can be found in the revised Renewal Assessment Report (final addendum) http://registerofquestions.efsa.europa.eu/roqFrontend/outputLoader?output=ON-4302

003606

From: Ross, Matthew
To: Rusyn, Ivan
Subject: Made it
Date: Wednesday, March 11, 2015 3:40:41 PM
Attachments: image001.png

Thanks, Ivan! I made my connecting flight with a few minutes to spare. Hope you made yours, too.

Let's keep in touch. You did a fantastic job as chair.

Best regards
Matt

On Mar 9, 2015, at 04:42, Rusyn, Ivan <[redacted]> wrote:

I would like to convene Group 4 downstairs in the first coffee break to discuss the information below.

Just to make sure we are all on the same page. Below are the evaluations from Groups 2 and 3 and the IARC matrix to get us to understand where our conclusions fit.

MAL: Human – Limited; Animal – sufficient → 2A; Group 4 evidence is strong to support carcinogenesis and we have data to show that the mechanisms can operate in humans, so we support the classification in 2A

DZN: Human – Limited; Animal – Inadequate (only one study) → 2B. Group 4 concludes that there is strong evidence for genotoxicity and oxidative stress and that these mechanisms can operate in humans. So we may consider upgrade to 2A.

GLY: Human – Limited; Animal – Limited → 2B. I have questions on the "limited" in animals as there are 2 studies showing significant effect... Nonetheless, Group 4 concludes that there is strong evidence for genotoxicity and oxidative stress and that these mechanisms can operate in humans. So we may consider upgrade to 2A.

<image001.png>

White, Dylan

From: Ross, Matthew
Sent: Monday, March 30, 2015 1:46 PM
To: Nathaniel Harmon
Subject: RE: Glyphosate Study Expertise Request

Hi Nathaniel,

I'm sorry but I don't have time to participate in the meeting. However, here are a couple of important points for your client to consider:

1. The international working group, convened by the IARC/WHO, that evaluated the 'carcinogenicity', or cancer-causing properties, of glyphosate earlier this month, did not conduct a *study*: instead, it considered all peer-reviewed scientific literature and publicly available government reports in their final form on the carcinogenicity of glyphosate and other pesticides.
2. The IARC deals with *hazard identification*. After a year-long process completed by an 8-day meeting, the Working Group provides a consensus classification as to the cancer causing effects of the exposure of interest. The classification indicates the strength of the evidence that a substance can cause cancer. It does not, however, conduct a *risk assessment* (i.e. defining the level of carcinogenic risk for individuals). This remains the responsibility of regulatory bodies, national and/or international, to take appropriate action to conduct such exercises.

   The distinction between hazard identification and risk assessment is an important one. I invite you to review the IARC preamble if you or your client would like more information: http://monographs.iarc.fr/ENG/Preamble/index.php.

Regards,

Matt Ross, PhD
Associate Professor
College of Veterinary Medicine
Mississippi State University

From: Nathaniel Harmon [redacted]
Sent: Monday, March 30, 2015 11:15 AM
To: Ross, Matthew
Subject: Glyphosate Study Expertise Request

Matthew,

I hope this message finds you well. I work for Guidepoint, a primary research company in New York, (www.guidepointglobal.com). Currently, we have a client, who is an institutional investor, and he is performing research and due diligence to better understand the recent study on glyphosate. Specifically, he is interested in speaking with experts to get an overview of the study and what the next steps from here are. I came across your expertise online and, considering your background, thought you would be a great resource for this project. I am reaching out to you to see if you may be interested in speaking with our client as part of a one-on-one paid consulting project.

Guidepoint is an independent research firm that connects our clients with industry professionals such as you. Calls typically last 45 min – 1hr, and we would compensate you for your time, if appropriate. As a matter of policy, our clients will not be asking you to discuss your own company nor will you be asked to discuss any confidential or proprietary information.

If this is something you may be interested in, please take a moment to register in our Network of Advisors:

https://new.guidepointglobaladvisors.com/apply/expresslead?k=3ogHKfGNqj1KMfVl.Y1sSkbAvx3pZmoZ2oHaF5Cs&r=1

This will allow us to arrange you on consultations with our Clients and allow you to invoice us for your time on the phone.

Please let me know if you have any questions regarding the project, the process, or my firm.

Best regards,

GUIDEPOINT

Nathaniel Harmon | Research Analyst
730 3rd Ave, 11th Floor | New York, NY 10017
[redacted]

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Key Characteristics of Carcinogens as a Basis for Organizing Data on Mechanisms of Carcinogenesis

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Key Characteristics of Carcinogens as a Basis for Organizing Data on Mechanisms of Carcinogenesis

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Berkeley, California 94720-7356 USA. [redacted]

Running title: Characteristic properties of human carcinogens

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Disclaimers: This paper does not necessary reflect the views and policies of the U.S. Environmental Protection Agency. Mention of trade names does not constitute endorsement or recommendation for use.

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Abstract

Background: A recent review by the International Agency for Research on Cancer (IARC) updated the assessments of the more than 100 agents classified as Group 1, carcinogenic to humans (IARC Monographs Volume 100, parts A-F). This exercise was complicated by the absence of a broadly accepted, systematic method for evaluating mechanistic data to support conclusions regarding human hazard from exposure to carcinogens.

Objectives and Methods: IARC therefore convened two workshops in which an international Working Group of experts identified 10 key characteristics, one or more of which are commonly exhibited by established human carcinogens.

Discussion: These characteristics provide the basis for an objective approach to identifying and organizing results from pertinent mechanistic studies. The ten characteristics are the abilities of an agent to: (1) act as an electrophile either directly or after metabolic activation; (2) be genotoxic; (3) alter DNA repair or cause genomic instability; (4) induce epigenetic alterations; (5) induce oxidative stress; (6) induce chronic inflammation; (7) be immunosuppressive; (8) modulate receptor-mediated effects; (9) cause immortalization; and (10) alter cell proliferation, cell death, or nutrient supply.

Conclusion: We describe the use of the 10 key characteristics to conduct a systematic literature search focused on relevant endpoints and construct a graphical representation of the identified mechanistic information. Next, we use benzene and polychlorinated biphenyls as examples to illustrate how this approach may work in practice. The approach described is similar in many respects to those currently being implemented by the U.S. EPA's IRIS Program and the U.S. National Toxicology Program.

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Introduction

Recently, the International Agency for Research on Cancer (IARC) completed a review of all its Group 1 human carcinogens and updated information on tumor sites and mechanisms of carcinogenesis (IARC Monograph Volume 100A-F). About half of the agents classified in Group 1 had been last reviewed more than 25 years ago, before mechanistic studies became prominent in evaluations of carcinogenicity. In addition, more recent studies have demonstrated that many cancer hazards reported in earlier studies were later observed to also cause cancer in other organs or through different exposure scenarios (Cogliano et al. 2011).

In compiling and updating the information for Volume 100A-F, two overarching issues became apparent. First, no broadly accepted systematic method for identifying, organizing, and summarizing mechanistic data for the purpose of decision-making in cancer hazard identification was readily available. Second, the agents documented and listed as human carcinogens showed a number of characteristics that are shared among many carcinogenic agents. Many human carcinogens act via multiple mechanisms causing various biological changes in the multistage process of carcinogenesis. Indeed, cancer was once described by reference to causative agents, with multistage development of tumors being characterized through the impact of particular chemicals described as initiators and promoters of cancer. Subsequently, multistage development of cancer was identified with morphological change being correlated with genetic alterations. The more recent description by Hanahan and Weinberg of hallmarks of cancer is not predicated on morphology or the impact of carcinogens, but on changes in gene expression and cell signaling (Hanahan and Weinberg 2011). These hallmarks are the properties of cancer cells and neoplasms, and are not characteristic of the agents that cause cancer. Tumors attributable to

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chemical carcinogens may be distinct by mutational analysis (Westcott et al, 2015), but all neoplasms exhibit the hallmarks. A recent computational toxicology study has shown that chemicals that alter the targets or pathways among the hallmarks of cancer are likely to be carcinogenic (Kleinstreuer et al. 2013). In addition, a series of reviews in *Carcinogenesis* by members of the Halifax Project Task Force utilized the hallmarks framework to identify the carcinogenic potential of low doses and mixtures of chemicals (Harris 2015).

In 2012, participants at two workshops convened by the IARC in Lyon, France extensively debated the mechanisms by which agents identified as human carcinogens (Group 1) produce cancer. The participants concluded that these carcinogens frequently exhibit one or more of 10 key characteristics (Table 1). Herein we describe these 10 key characteristics and discuss their importance in carcinogenesis. These characteristics are properties that human carcinogens commonly show and can encompass many different types of mechanistic endpoints. They are not mechanisms in and of themselves nor are they adverse outcome pathways.

Further, we describe how the 10 key characteristics can provide a basis for systematically identifying, organizing, and summarizing mechanistic information as part of the carcinogen evaluation process. The U.S. Environmental Protection Agency (EPA) and the National Toxicology Program (NTP) in the U.S., as well as the IARC internationally, have recognized a need for such an approach (Rooney et al. 2014). The U.S. National Research Council emphasized the need for consistent, transparent, systematic approaches for the identification, evaluation, and integration of data in EPA's IRIS assessments of carcinogens and elsewhere in human health hazard assessments (NRC 2014).

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Progress in the systematic evaluation of published evidence on the adverse health effects of environmental agents has been made through application of methods developed by evidence-based medicine (Koustas et al. 2014). However, mechanistic study databases present a challenge to systematic reviews in that the studies are typically both numerous and diverse, reporting on a multitude of endpoints and toxicity pathways. One recent example of a systematic approach searched for studies on endpoints relevant to nine cancer-related mechanistic categories in identifying and presenting mechanistic evidence on di(2-ethylhexyl)phthalate, a chemical with a complex database of over 3000 research papers (Kushman et al. 2013). In this publication, the categories of mechanistic evidence were identified from a compendium of published reviews. This approach may be difficult to translate to agents with controversial or limited mechanistic evidence. It also would not permit comparisons across agents, including attempts to understand similarities or differences with human carcinogens. Further, it may be biased against the most recent mechanistic and molecular epidemiology studies that have not been the subject of a prior expert review.

To facilitate a systematic and uniform approach to organizing mechanistic data relevant to carcinogens, we propose the use of 10 key characteristics of human carcinogens as a basis for identifying and categorizing scientific findings relevant to cancer mechanisms when assessing whether an agent is a potential human carcinogen. A significant advantage of this approach is that it would encompass a wide range of endpoints of known relevance to carcinogenesis as identified through examination of the IARC Monographs on Group 1 carcinogens. Mechanistic topics can be included regardless of whether they have been the subject of prior expert reviews of any particular chemical. This should introduce objectivity that could reduce reliance on expert opinion, as well as facilitate comparisons across agents. Moreover, at its essence, the approach

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may afford a broad consideration of the mechanistic evidence rather than focusing narrowly on independent mechanistic hypotheses or pathways in isolation.

Herein, we demonstrate the applicability of this proposed systematic strategy for searching and organizing the literature using benzene and polychlorinated biphenyls (PCBs) as examples. The mechanistic study database for both of these chemicals is large, comprising over 1,800 studies for benzene and almost 3,900 for PCBs, many with multiple mechanistic endpoints. We conducted systematic literature searches for endpoints pertinent to the 10 key characteristics of human carcinogens, utilizing literature trees to indicate the human and experimental animal studies that reported endpoints relevant to each characteristic. To further indicate their potential contribution to benzene and PCB carcinogenesis, we organized the characteristics into a graphical network representative of an overall mechanistic pathway.

Two recent IARC Monographs (Guyton et al. 2015; Loomis et al. 2015) have applied the 10 key characteristics described here for a variety of agents and also organized the results into graphical networks. Overall, this categorization facilitated objective consideration of the relevant mechanistic information, thereby advancing analyses of hypothesized mechanisms and toxicity pathways. Because mechanistic data may provide evidence of carcinogenicity, and can play a role in up- or downgrading an evaluation based on cancer findings in animals, we suggest that this systematic approach to organizing the available data will assist future IARC Working Groups and other agencies in evaluating agents as potential human carcinogens especially in the absence of convincing epidemiological data on cancer in humans.

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Description of the Key Characteristics of Carcinogens

The number of ways by which agents contribute to carcinogenesis can be extensive if all biochemical or molecular endpoints are considered. However, these mechanisms can be grouped into a limited number of categories (e.g., genotoxicity, immunosuppression, etc.). Guyton and coworkers described 15 types of "key events" associated with human carcinogens that collectively represented many carcinogenic mechanisms (Guyton et al. 2009). The experts present at the first of the IARC meetings in 2012 originally identified 24 mechanistic endpoints with several subcategories in each. This number of endpoints was considered too impractical as a guide for categorizing the literature, and the Working Group merged these categories into 10 at the second meeting in 2012, concluding that human carcinogens commonly show one or more of the 10 key characteristic properties listed in Table 1. These represent the majority of established properties of human carcinogens as described below.

Characteristic 1: Is Electrophilic or Can Be Metabolically Activated to Electrophiles

Electrophiles are electron-seeking molecules that commonly form addition products, commonly referred to as adducts, with cellular macromolecules including DNA, RNA, lipids and proteins. Some chemical carcinogens are direct-acting electrophiles, whereas others require chemical conversion within the body (Salnikow and Zhitkovich 2008), or biotransformation by enzymes in a process termed metabolic activation (Miller 1970). Examples of direct-acting electrophilic carcinogens include sulfur mustards and ethylene oxide (Batal et al. 2014; Grosse et al. 2007; IARC 2008; Rusyn et al. 2005). The classic examples of chemical agents that require metabolic activation to become carcinogenic include polycyclic aromatic hydrocarbons, aromatic amines, *N*-nitrosamines, aflatoxins and benzene, which by themselves are relatively inert (Slaga et al.

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1980; Smith 1996). A number of enzymes, including cytochrome P450s, flavin mono-oxygenase, prostaglandin synthase and various peroxidases, can biotransform relatively inert chemical compounds to potent toxic and carcinogenic metabolites or reactive intermediates (Hecht 2012; O'Brien 2000). The ability to form adducts on nucleic acids and proteins is a common property of these inherently electrophilic and/or metabolically activated human carcinogens (Ehrenberg 1984).

Characteristic 2: Is Genotoxic

The term genotoxic (Ehrenberg 1973) refers to an agent that induces DNA damage, mutation, or both. DNA damage can be spontaneous in origin through errors of nucleic acid metabolism or can be induced by endogenous or exogenous agents. In some cases the exogenous agents may also be generated endogenously, such as formaldehyde and acetaldehyde, producing a background level of DNA damage. Examples of DNA damage include DNA adducts (a molecule bound covalently to DNA), DNA strand breaks (breaks in the phosphodiester bonds), DNA crosslinks, and DNA alkylation. DNA damage by itself is not a mutation and generally does not alter the linear sequence of nucleotides (or bases) in the DNA, whereas a mutation is a change in the DNA sequence and usually arises as the cell attempts to repair the DNA damage (Shaughnessy 2009).

Mutations can be classified into three groups based on their location or involvement in the genome. Gene or point mutations are changes in nucleotide sequence within a gene (e.g., base substitutions, frameshifts, and small deletions/duplications). Chromosomal mutations are changes in nucleotide sequence that extend over multiple genes (e.g., chromosome aberrations, translocations, large deletions, duplications, insertions, inversions, or micronuclei due to

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chromosome breakage). Genomic mutations involve the duplication or deletion of nucleotide sequences of an entire chromosome, an example of which is aneuploidy or formation of micronuclei that contain a centromere. A large proportion of Group 1 carcinogens are genotoxic, as documented in IARC Monographs Volume 100 A-F (http://monographs.iarc.fr/ENG/Monographs/PDFs/index.php).

Characteristic 3: Alters DNA Repair or Causes Genomic Instability

Normal cells avoid deleterious mutations by replicating their genomes with high accuracy. However, the fidelity of DNA replication can vary widely depending on the DNA polymerase involved, introducing the possibility of error. Indeed, most spontaneous mutations are caused by polymerase error (Preston et al. 2010). The nature of the error, the flanking sequence, the presence of DNA damage and the ability to correct errors, all impact on the outcome of this process (Arana and Kunkel 2010). As a consequence, defects in processes that determine DNA-replication fidelity can confer strong mutator phenotypes that result in genomic instability. Thus, carcinogens may act not only by producing DNA damage directly, but also by altering the processes that control normal DNA replication or repair of DNA damage. Examples include the inhibition of DNA repair by cadmium (Candeias et al. 2010) and formaldehyde (Luch et al. 2014).

Genomic instability is a well-recognized feature of many cancers (Bielas et al. 2006) and considered to be one of the enabling characteristics of cancer (Hanahan and Weinberg 2011). Cells exposed to ionizing radiation have genetic instability that is a relatively late-occurring event that appears several cell generations after irradiation and results in a reduced ability to

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replicate the genotype faithfully (Kadhim et al. 2013). The events indicating genomic instability include chromosome aberrations, gene mutations, microsatellite instability, and apoptosis. These events are observed after exposure to arsenic (Bhattacharjee et al. 2013) and cadmium (Filipic 2012).

Characteristic 4: Induces Epigenetic Alterations

The term "epigenetic" refers to stable changes in gene expression and chromatin organization that are not caused by changes in the DNA sequence itself and can be inherited over cell divisions (Herceg et al. 2013). Epigenetic phenomena, including changes to the DNA methylome and chromatin compaction states, along with histone modification can impact the carcinogenic process by affecting gene expression and DNA repair dynamics (Herceg et al. 2013). A wide range of carcinogens have been shown to deregulate the epigenome, and it has been suggested that their mechanism may involve disruption of epigenetic mechanisms (Pogribny and Rusyn 2013). However, evidence for a causal role of epigenetic changes in cancer caused by Group 1 agents was considered to be limited in Volume 100, and for many agents, their impact on the epigenome was considered to be a secondary mechanism of carcinogenesis (Herceg et al. 2013). Herceg and others (Herceg et al. 2013) have described a wealth of studies demonstrating the impact of carcinogens on epigenetic mechanisms. They note, however, that most carcinogens (even those reviewed for Volume 100 in 2008 and 2009) were evaluated by IARC Working Groups before new data on their epigenetic effects became available. This evolving area will generate new mechanistic data in the years to come.

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Characteristic 5: Induces Oxidative stress

Many carcinogens are capable of influencing redox balance within target cells. If an imbalance occurs, favoring formation of reactive oxygen and/or nitrogen species at the expense of their detoxification, this is referred to as oxidative stress. Reactive oxygen species and other free radicals arising from tissue inflammation, xenobiotic metabolism, interruption of mitochondrial oxidative phosphorylation (Figueira et al. 2013), or reduced turnover of oxidized cellular components may play key roles in many of the processes necessary for the conversion of normal cells to cancer cells. However, oxidative stress is not unique to cancer induction and is associated with a number of chronic diseases and pathological conditions, e.g., cardiovascular disease (Kayama et al. 2015), neurodegenerative disease (Chen et al. 2015), and chronic inflammation (Suman et al. 2015). Oxidative stress is also a common occurrence in neoplastic tissue and can be part of the tumor environment (Suman et al. 2015).

Oxidative damage is considered a major factor in the generation of mutations in DNA and over 100 different types of oxidative DNA damage have been identified (Klaunig et al. 2011). At least 24 base modifications are produced by reactive oxygen species, as well as DNA-protein crosslinks and other lesions (Berquist and Wilson 2012), all potentially leading to genomic instability. Oxidative damage to DNA can lead to point mutations, deletions, insertions, or chromosomal translocations, which may cause oncogene activation and tumor suppressor gene inactivation, and potentially initiate or promote carcinogenesis (Berquist and Wilson 2012; Klaunig et al. 2011). Thus, the induction of oxygen radical-induced cellular injury is a characteristic of a set of diverse carcinogens, including radiation, asbestos, and carcinogenic infectious agents.

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Characteristic 6: Induces Chronic Inflammation

Chronic inflammation from persistent infections, such as that caused by *H. pylori*, as well as that produced by chemical agents including silica or asbestos fibers, has been associated with several forms of cancer (Grivennikov et al. 2010). Indeed, inflammation has been hypothesized to contribute to multiple aspects of cancer development and progression (Trinchieri 2012) and is an enabling hallmark of cancer (Hanahan and Weinberg 2011). Inflammation acts by both intrinsic and extrinsic pathways. Persistent infection and chronic inflammation disrupt local tissue homeostasis and alter cell signaling, leading to the recruitment and activation of inflammatory cells. These constitute extrinsic pathways linking inflammation to cancer (Multhoff and Radons 2012). On the other hand, intrinsic pathways driven by activation of proto-oncogenes in pre-neoplastic and neoplastic cells recruit host-derived inflammatory cells that accelerate tumor promotion and progression (Grivennikov et al. 2010). Because strong links exist between inflammation and the induction of oxidative stress and genomic instability, it may be difficult to separate out the importance of each of these mechanisms.

Characteristic 7: Is Immunosuppressive

Immunosuppression is a reduction in the capacity of the immune system to respond effectively to foreign antigens, including antigens on tumor cells. Persistent immunosuppression presents a risk of cancer, especially excess risk for lymphoma. For example, immunosuppression poses a significant risk when it is accompanied by continuing exposure to foreign antigens, such as in people with organ transplants, or when it occurs in individuals who are latently infected with a carcinogenic virus (Hartge and Smith 2007; Smith et al. 2004). Immune suppression differs from other mechanisms of carcinogenesis in that agents that cause immunosuppression may not

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directly transform normal cells into potential tumor cells. Potentially neoplastic cells that arise naturally, or that have been transformed by other carcinogens acting by a mechanism such as genotoxicity or by the various mechanisms of action associated with carcinogenic viruses, escape immune surveillance in immunosuppressed individuals. As a result, survival of these cells and their replication to form tumors is greatly facilitated by immune suppression. Several carcinogens act entirely or largely by immunosuppression, often in concert with other Group 1 agents, especially oncogenic infectious agents. The Group 1 agents that act by immunosuppression include Human Immunodeficiency Virus (HIV-1) and the immunosuppressive drug cyclosporin (Rafferty et al. 2012).

Characteristic 8: Modulates Receptor-mediated effects

Numerous carcinogens act as ligands to receptor proteins, including menopausal hormone therapy, 2,3,7,8-tetrachlorodibenzo-para-dioxin and PCBs (Wallace and Redinbo 2013). Receptor-mediated activation broadly falls into two categories: (a) intracellular activation, mediated by nuclear receptors that translocate into the nucleus and act on DNA as transcription factors (Aranda and Pascual 2001); and (b) activation of cell surface receptors that induce signal-transduction pathways resulting in biological responses that involve a variety of protein kinases (Griner and Kazanietz 2007). Most exogenous agents act as agonists by competing for binding with an endogenous ligand; however, there are also receptors for which few or no endogenous ligands have been identified, such as the aryl-hydrocarbon (Ah) receptor (Baek and Kim 2014; Ma 2011). Receptor-mediated activation most often results in changes in gene transcription. Molecular pathways that are regulated through ligand-receptor interaction and are most relevant to carcinogenesis include cell proliferation (e.g., stimulation of the normal proliferative pathways

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as is the case for estrogen-dependent tissues and hormone therapy), xenobiotic metabolism, apoptosis, as well as modulation of the bioavailability of endogenous ligands by affecting biosynthesis, bioactivation, and degradation (Rushmore and Kong 2002).

Characteristic 9: Causes Immortalization

Several human DNA and RNA viruses, including various human papillomaviruses, Epstein-Barr virus, Kaposi's sarcoma-associated herpesvirus, hepatitis B virus, hepatitis C virus, and human immunodeficiency virus, are carcinogenic to humans (Bouvard et al. 2009). These viruses have evolved multiple molecular mechanisms to disrupt specific cellular pathways to facilitate aberrant replication. Although oncogenic viruses belong to different families, their strategies in human cancer development show many similarities and involve viral-encoded oncoproteins targeting the key cellular proteins that regulate cell growth (Saha et al. 2010). Recent studies show that virus and host interactions also occur at the epigenetic level (Allday 2013). The result of these viral effects is to immortalize the target tissue cells such that they are not subject to the Hayflick limit, the point at which cells can no longer divide due to DNA damage or shortened telomeres (Klingelhutz 1999). For example, the Human Papillomavirus type-16 (HPV-16) *E6* and *E7* oncogenes are selectively retained and expressed in cervical carcinomas, and expression of *E6* and *E7* is sufficient to immortalize human cervical epithelial cells (Yugawa and Kiyono 2009).

Characteristic 10: Alters Cell Proliferation, Cell Death or Nutrient Supply

There are at least three scenarios related to carcinogenesis in which alterations in cellular replication and/or cell-cycle control have been described. One invokes the predisposition for

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unrepaired DNA damage leading to cancer-initiating mutations in replicating cells, another has attempted to identify sustained replication as a key mechanistic event, and a third describes the ability of a transformed cell to escape normal cell-cycle control and to continue replication. A component common to all three scenarios is the evasion of apoptosis or other terminal programming, including autophagy, in at least a proportion of the cell population (Ryter et al. 2014).

Necrotic cell death releases pro-inflammatory signals into the surrounding tissue microenvironment, recruiting inflammatory immune cells to the site of trauma, which can enhance cancer-cell proliferation and promote cancer metastasis (Coussens and Pollard 2011; Coussens et al. 2013; Pollard 2008). In contrast, various forms of apoptosis and autophagy (Galluzzi et al. 2015) have the opposite effect by removing potentially cancerous cells from a population before they acquire the changes permitting malignancy. Many agents affect necrosis, apoptosis and/or autophagy and can have profoundly divergent effects on cancer induction in different tissues.

In addition to cell death caused directly by agent toxicity, cells may die within a tumor as a result of an impaired nutrient supply. Neoplastic cell numbers can increase exponentially, quickly outstripping the supply capabilities of the existing tissue vasculature. Neoangiogenesis, in which new blood vessels grow into a tumor, is key to providing this supply of nutrients. Thus, agents that promote or inhibit angiogenesis will promote or delay tumor growth (Hu et al. 2015).

Cancer cells also usually show quite different cellular energetics, relying on glycolysis for energy even under aerobic conditions (Rajendran et al. 2004). Although a likely consequence of mutation and altered gene expression rather than a cancer-inducing mechanism, any modification

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of cellular energetics may reflect an important cancer-relevant switch in the cell or tissue's metabolic state.

Using the key characteristics to systematically identify, organize, and summarize mechanistic information

Step 1: Identifying the relevant information

The starting point for systematic evaluation is to conduct comprehensive searches of the peer-reviewed literature aimed at identifying mechanistic data (Kushman et al. 2013). The searches can be constructed to address a series of study questions in the PECO (population, exposure, comparator, and outcomes) framework (Higgins and Green 2011) wherein endpoints associated with the key characteristics are identified. Specifically, the questions to be answered by the searches are, "Does exposure to the agent induce endpoints associated with one or more specific key characteristic properties of carcinogens"? The population (humans and any relevant experimental systems), exposure (the agent and relevant metabolites) and comparator (the unexposed comparison group or condition) should be sufficiently broad to identify a range of available mechanistic data informative of the overall evaluation of carcinogenic hazard. This approach thus entails comprehensive, targeted literature searches using appropriate Medical Search Heading (MeSH) terms and key words to identify evidence on the 10 key characteristics for the agent(s) or exposure(s) under evaluation.

Additional complementary literature searches may incorporate terms for the agent and its metabolites, alone or in combination with broad terms for carcinogenicity or related effects. For instance, because US EPA Integrated Risk Information System (IRIS) toxicological reviews also

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encompass a range of non-cancer toxicities, "top-down" broad literature searches aimed at comprehensively identifying studies on all potential toxic effects of an agent are employed (EPA 2014; NRC 2014). These comprehensive searches of peer-reviewed literature are supplemented by examining past IARC Monographs or other authoritative reviews; databases (e.g., PubChem); and, peer-reviewed government reports can also be systematically searched. The search terms used and literature retrieved can be documented (e.g., using MyNCBI, which saves searches of the National Center for Biotechnology database, or https://hawcproject.org).

Step 2: Screening and organizing the results

Based on title and abstract review, studies identified initially are excluded if no data on the chemical or a metabolite are reported, or if no data on toxicological or other cancer-related effects of the chemical is provided. For example, a study on levels of a chemical, but not effects of the chemical, would be excluded. Included studies are then organized by the population (human or experimental systems) and by the endpoints associated with the 10 key characteristics (see Table 1). Studies relevant to toxicokinetics (covering absorption, distribution, metabolism and excretion) are also identified. Additionally, authoritative, comprehensive review articles are identified, as are studies reporting toxicological endpoints in cancer target and non-target tissues. These may include morphological evaluations pertaining to the dysfunction of organs, tissues, and cells. Importantly, studies reporting endpoints that are relevant to multiple characteristics may fall under several categories.

To illustrate these two steps, targeted literature searches were conducted to identify endpoints for the effects of benzene pertinent to the 10 key characteristics, in populations comprising humans or experimental systems. The literature searches were conducted using the Health Assessment

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Workplace Collaborative (HAWC) Literature Search tool (https://hawcproject.org/), documenting the search terms, sources, and articles retrieved. Following title and abstract review, studies were excluded if they were not about benzene or its metabolites, or if they reported no data on toxicological endpoints. Included studies were further sorted into categories representing the 10 key characteristics based on the mechanistic endpoints and species evaluated (i.e. human in vivo, human in vitro, mammalian in vivo, mammalian in vitro, non-mammalian; see Figure 1). The figure also identifies reviews, gene expression studies, and articles relevant to toxicokinetics, toxicity, or susceptibility.

Step 3: Using the key characteristics to synthesize mechanistic information and to develop adverse-outcome networks

It is increasingly evident that multiple biological alterations or sets of different perturbations are necessary to convert a normal cell to a transformed cell and ultimately a tumor (Hanahan and Weinberg 2011). Carcinogens appear to impact this complex process in various ways and can act through multiple mechanisms to induce cancer and other adverse health outcomes (Goodson et al. 2015; Guyton et al. 2009). Using the 10 key characteristics as a basis, the collected information can be organized to form hypotheses and evaluate the evidentiary support for mechanistic events as a function of relevant aspects (e.g. dose, species, temporality, etc) (Guyton et al. 2009). The diverse and complex mechanistic endpoints elicited by benzene can then be organized into an overview inclusive of multiple alterations and any linkages thereof (Figure 2). The resulting overview can provide guidance for further assessments of the literature, including dose relevance, species relevance, and temporality of events. This additional detailed information can then be used to produce proposed mechanisms or adverse outcome pathway networks as

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described in (McHale et al. 2012) and the EPA's NexGen Risk Assessment Report (EPA 2014). We note that there is evidence that benzene is associated with 8 of the 10 key characteristics we have described.

Figure 3 presents a similar overview for PCBs based on data from IARC Monograph Volume 107 (IARC 2015). In summarizing the mechanistic evidence, this Monograph Working Group indicated that PCBs may induce up to 7 of the 10 key characteristics in producing carcinogenicity (Lauby-Secretan et al. 2013). We note that the less chlorinated PCBs are associated with key characteristics similar to benzene (metabolic activation, DNA damage, cellular proliferation), whereas the dioxin-like PCBs are associated primarily with receptor-mediated activities.

Recently, using this same approach, the Working Groups of IARC Monograph Volume 112 and Volume 113 concluded that strong mechanistic evidence exists for 5 key characteristics being involved in malathion carcinogenicity (i.e. genotoxicity, oxidative stress, inflammation, receptor-mediated effects and cell proliferation or death), 3 in DDT carcinogenicity (i.e. immunosuppression, receptor-mediated effects and oxidative stress) and 2 each for diazinon and glyphosate (i.e. genotoxicity and oxidative stress), providing evidence to support their classification as probable human carcinogens in Group 2A (Guyton et al. 2015; Loomis et al. 2015).

Discussion and Conclusions

Identification and incorporation of important, novel scientific findings providing insights into cancer mechanisms is an increasingly essential aspect of carcinogen hazard identification and

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risk assessment. Systematic approaches are needed to organize the available mechanistic data relevant to the overall evaluation of the carcinogenic hazard of an agent. Information to support the identification of 10 key characteristics of human carcinogens was obtained during the Volume 100 Monographs and two subsequent expert workshops. These characteristics, although not necessarily representing mechanisms themselves, provide the rationale for an objective approach to identifying and organizing relevant mechanistic data. Using literature collected previously by others as well as by us, we have categorized the literature data according to the 10 characteristics for benzene and PCBs. This approach identified pertinent positive literature for 8 of the 10 key characteristics on benzene and 7 for PCBs, thereby providing a practical, objective method for organizing the large mechanistic literature associated with these chemicals.

This approach also lays the groundwork for a structured evaluation of the strength of the mechanistic evidence base, and therefore its utility in supporting hazard classifications. In the IARC Monographs the strength of the evidence that any carcinogenic effect observed is due to a particular mechanism is evaluated using the terms 'weak', 'moderate' or 'strong' (http://monographs.iarc.fr/ENG/Preamble/index.php). In general, the strongest indications that a particular mechanism operates in humans derive from data obtained in exposed humans or in human cells in vitro. Data from experimental animals can support a mechanism by findings of consistent results and from studies that challenge the hypothesized mechanism experimentally. Other considerations include whether multiple mechanisms might contribute to tumor development, whether different mechanisms might operate in different dose ranges, whether separate mechanisms might operate in humans and experimental animals and whether a unique mechanism might operate in a susceptible group. The possible contribution of alternative mechanisms must be considered before concluding that tumors observed in experimental animals

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are not relevant to humans. An uneven level of experimental support for different mechanisms may reflect that disproportionate resources have been focused on investigating a favored mechanism. All of these factors make assignment of descriptors such as 'strong' to the mechanistic evidence challenging, but recent experience with two IARC Monograph meetings suggest that the weighing of the evidence on the basis of the 10 key characteristics focuses the group discussion on the available science and allows rapid consensus to be reached regardless of the strength of the evidence base (Guyton et al. 2015; Loomis et al. 2015).

Because the literature search and categorization approach described herein is comprehensive, it may aid consideration of the overall strength of the mechanistic database according to these principles. In particular, it is inclusive of diverse mechanistic evidence, enabling support for divergent or related mechanisms from human and experimental systems to be identified. Moreover, the literature support for endpoints relevant to specific mechanisms can be evaluated in an integrated fashion when the mechanism is complex. Additionally, comparisons across agents will be facilitated, including evaluation of any similarities or differences in the pattern of key characteristics with agents that are currently classified.

As this approach is carried forward, we hope it will facilitate the objective identification of mechanistic data for consideration in the context of epidemiology, animal bioassay, or other types of evidence (e.g., studies in model organisms or *in vitro* assays) when classifying agents with regard to carcinogenic hazard. Equally important is to consider whether key characteristics of carcinogens are apparent upon exposures that are relevant to human health (Thomas et al. 2013). Overall, these developments will aid advancement of future evaluations of newly

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introduced chemicals, including those for which mechanistic data provide the primary evidence of carcinogenicity.

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REFERENCES

Allday MJ. 2013. Ebv finds a polycomb-mediated, epigenetic solution to the problem of oncogenic stress responses triggered by infection. Frontiers in genetics 4:212.

Arana ME, Kunkel TA. 2010. Mutator phenotypes due to DNA replication infidelity. Semin Cancer Biol 20:304-311.

Aranda A, Pascual A. 2001. Nuclear hormone receptors and gene expression. Physiological reviews 81:1269-1304.

Baek SH, Kim KI. 2014. Emerging roles of orphan nuclear receptors in cancer. Annual review of physiology 76:177-195.

Batal M, Boudry I, Mouret S, Clery-Barraud C, Wartelle J, Berard I, et al. 2014. DNA damage in internal organs after cutaneous exposure to sulphur mustard. Toxicology and applied pharmacology 278:39-44.

Berquist BR, Wilson DM, 3rd. 2012. Pathways for repairing and tolerating the spectrum of oxidative DNA lesions. Cancer Lett 327:61-72.

Bhattacharjee P, Banerjee M, Giri AK. 2013. Role of genomic instability in arsenic-induced carcinogenicity. A review. Environment international 53:29-40.

Bielas JH, Loeb KR, Rubin BP, True LD, Loeb LA. 2006. Human cancers express a mutator phenotype. Proc Natl Acad Sci U S A 103:18238-18242.

Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. 2009. A review of human carcinogens--part b: Biological agents. The Lancet Oncology 10:321-322.

Candeias S, Pons B, Viau M, Caillat S, Sauvaigo S. 2010. Direct inhibition of excision/synthesis DNA repair activities by cadmium: Analysis on dedicated biochips. Mutat Res 694:53-59.

Chen SH, Oyarzabal EA, Hong JS. 2015. Critical role of the mac1/nox2 pathway in mediating reactive microgliosis-generated chronic neuroinflammation and progressive neurodegeneration. Current opinion in pharmacology 26:54-60.

Cogliano VJ, Baan R, Straif K, Grosse Y, Lauby-Secretan B, El Ghissassi F, et al. 2011. Preventable exposures associated with human cancers. Journal of the National Cancer Institute 103:1827-1839.

Coussens LM, Pollard JW. 2011. Leukocytes in mammary development and cancer. Cold Spring Harbor perspectives in biology 3.

Environ Health Perspect DOI: 10.1289/ehp.1509912
Advance Publication: Not Copyedited

Coussens LM, Zitvogel L, Palucka AK. 2013. Neutralizing tumor-promoting chronic inflammation: A magic bullet? Science 339:286-291.

Ehrenberg L. 1984. Covalent binding of genotoxic agents to proteins and nucleic acids. IARC scientific publications:107-114.

Ehrenberg L, P. Brooks, H. Druckrey, B. Langerlöf, J. Litwin, G. Williams 1973. Report of group 3. Vol. 3:AMBIO Special Report, 15-16.

EPA US. 2014. Next generation risk assessment: Incorporation of recent advances in molecular, computational, and systems biology. EPA/600/R-14/004. Washington, DC:U.S. Environmental Protection Agency.

Figueira TR, Barros MH, Camargo AA, Castilho RF, Ferreira JC, Kowaltowski AJ, et al. 2013. Mitochondria as a source of reactive oxygen and nitrogen species: From molecular mechanisms to human health. Antioxidants & redox signaling 18:2029-2074.

Filipic M. 2012. Mechanisms of cadmium induced genomic instability. Mutat Res 733:69-77.

Galluzzi L, Bravo-San Pedro JM, Vitale I, Aaronson SA, Abrams JM, Adam D, et al. 2015. Essential versus accessory aspects of cell death: Recommendations of the nccd 2015. Cell death and differentiation 22:58-73.

Goodson WH, 3rd, Lowe L, Carpenter DO, Gilbertson M, Manaf Ali A, Lopez de Cerain Salsamendi A, et al. 2015. Assessing the carcinogenic potential of low-dose exposures to chemical mixtures in the environment: The challenge ahead. Carcinogenesis 36 Suppl 1:S254-296.

Griner EM, Kazanietz MG. 2007. Protein kinase c and other diacylglycerol effectors in cancer. Nature reviews Cancer 7:281-294.

Grivennikov SI, Greten FR, Karin M. 2010. Immunity, inflammation, and cancer. Cell 140:883-899.

Grosse Y, Baan R, Straif K, Secretan B, El Ghissassi F, Bouvard V, et al. 2007. Carcinogenicity of 1,3-butadiene, ethylene oxide, vinyl chloride, vinyl fluoride, and vinyl bromide. The Lancet Oncology 8:679-680.

Guyton KZ, Kyle AD, Aubrecht J, Cogliano VJ, Eastmond DA, Jackson M, et al. 2009. Improving prediction of chemical carcinogenicity by considering multiple mechanisms and applying toxicogenomic approaches. Mutat Res 681:230-240.

Environ Health Perspect DOI: 10.1289/ehp.1509912
Advance Publication: Not Copyedited

Guyton KZ, Loomis D, Grosse Y, El Ghissassi F, Benbrahim-Tallaa L, Guha N, et al. 2015. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. The Lancet Oncology 16:490-491.

Hanahan D, Weinberg RA. 2011. Hallmarks of cancer: The next generation. Cell 144:646-674.

Harris CC. 2015. Cause and prevention of human cancer. Carcinogenesis 36 Suppl 1:S1.

Hartge P, Smith MT. 2007. Environmental and behavioral factors and the risk of non-hodgkin lymphoma. Cancer Epidemiol Biomarkers Prev 16:367-368.

Hecht SS. 2012. Lung carcinogenesis by tobacco smoke. Int J Cancer 131:2724-2732.

Herceg Z, Lambert MP, van Veldhoven K, Demetriou C, Vineis P, Smith MT, et al. 2013. Towards incorporating epigenetic mechanisms into carcinogen identification and evaluation. Carcinogenesis 34:1955-1967.

Higgins JPT, Green S. 2011. Cochrane handbook for systematic reviews of interventions, version 5.1.0. .

Hu Z, Brooks SA, Dormoy V, Hsu CW, Hsu HY, Lin LT, et al. 2015. Assessing the carcinogenic potential of low-dose exposures to chemical mixtures in the environment: Focus on the cancer hallmark of tumor angiogenesis. Carcinogenesis 36 Suppl 1:S184-202.

IARC. 2008. Iarc monographs on the evaluation of carcinogenic risks to humans. Volume 97. 1,3-butadiene, ethylene oxide and vinyl halides (vinyl fluoride, vinyl chloride and vinyl bromide). IARC monographs on the evaluation of carcinogenic risks to humans / World Health Organization, International Agency for Research on Cancer 97:3-471.

Kadhim M, Salomaa S, Wright E, Hildebrandt G, Belyakov OV, Prise KM, et al. 2013. Non-targeted effects of ionising radiation-implications for low dose risk. Mutat Res 752:84-98.

Kayama Y, Raaz U, Jagger A, Adam M, Schellinger IN, Sakamoto M, et al. 2015. Diabetic cardiovascular disease induced by oxidative stress. International journal of molecular sciences 16:25234-25263.

Klaunig JE, Wang Z, Pu X, Zhou S. 2011. Oxidative stress and oxidative damage in chemical carcinogenesis. Toxicology and applied pharmacology 254:86-99.

Klingelhutz AJ. 1999. The roles of telomeres and telomerase in cellular immortalization and the development of cancer. Anticancer research 19:4823-4830.

Environ Health Perspect DOI: 10.1289/ehp.1509912
Advance Publication: Not Copyedited

Koustas E, Lam J, Sutton P, Johnson PI, Atchley DS, Sen S, et al. 2014. The navigation guide - evidence-based medicine meets environmental health: Systematic review of nonhuman evidence for pfoa effects on fetal growth. Environ Health Perspect 122:1015-1027.

Kushman ME, Kraft AD, Guyton KZ, Chiu WA, Makris SL, Rusyn I. 2013. A systematic approach for identifying and presenting mechanistic evidence in human health assessments. Regulatory toxicology and pharmacology : RTP 67:266-277.

Lauby-Secretan B, Loomis D, Grosse Y, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, et al. 2013. Carcinogenicity of polychlorinated biphenyls and polybrominated biphenyls. The Lancet Oncology 14:287-288.

Loomis D, Guyton K, Grosse Y, El Ghissasi F, Bouvard V, Benbrahim-Tallaa L, et al. 2015. Carcinogenicity of lindane, ddt, and 2,4-dichlorophenoxyacetic acid. The Lancet Oncology.

Luch A, Frey FC, Meier R, Fei J, Naegeli H. 2014. Low-dose formaldehyde delays DNA damage recognition and DNA excision repair in human cells. PloS one 9:e94149.

Ma Q. 2011. Influence of light on aryl hydrocarbon receptor signaling and consequences in drug metabolism, physiology and disease. Expert opinion on drug metabolism & toxicology 7:1267-1293.

McHale CM, Zhang L, Smith MT. 2012. Current understanding of the mechanism of benzene-induced leukemia in humans: Implications for risk assessment. Carcinogenesis 33:240-252.

Miller JA. 1970. Carcinogenesis by chemicals: An overview--g. H. A. Clowes memorial lecture. Cancer Res 30:559-576.

Multhoff G, Radons J. 2012. Radiation, inflammation, and immune responses in cancer. Front Oncol 2:58.

NRC. 2014. Review of epa's integrated risk information system (iris) process. Washington, DC:National Research Council.

O'Brien PJ. 2000. Peroxidases. Chem Biol Interact 129:113-139.

Pogribny IP, Rusyn I. 2013. Environmental toxicants, epigenetics, and cancer. Advances in experimental medicine and biology 754:215-232.

Pollard JW. 2008. Macrophages define the invasive microenvironment in breast cancer. Journal of leukocyte biology 84:623-630.

Preston BD, Albertson TM, Herr AJ. 2010. DNA replication fidelity and cancer. Semin Cancer Biol 20:281-293.

Environ Health Perspect DOI: 10.1289/ehp.1509912
Advance Publication: Not Copyedited

Rafferty P, Egenolf D, Brosnan K, Makropoulos D, Jordan J, Meshaw K, et al. 2012. Immunotoxicologic effects of cyclosporine on tumor progression in models of squamous cell carcinoma and b-cell lymphoma in c3h mice. Journal of immunotoxicology 9:43-55.

Rajendran JG, Mankoff DA, O'Sullivan F, Peterson LM, Schwartz DL, Conrad EU, et al. 2004. Hypoxia and glucose metabolism in malignant tumors: Evaluation by [18f]fluoromisonidazole and [18f]fluorodeoxyglucose positron emission tomography imaging. Clinical cancer research : an official journal of the American Association for Cancer Research 10:2245-2252.

Rooney AA, Boyles AL, Wolfe MS, Bucher JR, Thayer KA. 2014. Systematic review and evidence integration for literature-based environmental health science assessments. Environ Health Perspect 122:711-718.

Rushmore TH, Kong AN. 2002. Pharmacogenomics, regulation and signaling pathways of phase i and ii drug metabolizing enzymes. Current drug metabolism 3:481-490.

Rusyn I, Asakura S, Li Y, Kosyk O, Koc H, Nakamura J, et al. 2005. Effects of ethylene oxide and ethylene inhalation on DNA adducts, apurinic/apyrimidinic sites and expression of base excision DNA repair genes in rat brain, spleen, and liver. DNA repair 4:1099-1110.

Ryter SW, Mizumura K, Choi AM. 2014. The impact of autophagy on cell death modalities. International journal of cell biology 2014:502676.

Saha A, Kaul R, Murakami M, Robertson ES. 2010. Tumor viruses and cancer biology: Modulating signaling pathways for therapeutic intervention. Cancer biology & therapy 10:961-978.

Salnikow K, Zhitkovich A. 2008. Genetic and epigenetic mechanisms in metal carcinogenesis and cocarcinogenesis: Nickel, arsenic, and chromium. Chemical research in toxicology 21:28-44.

Shaughnessy DT, DeMarini, D. M. . 2009. Types and consequences of DNA damage. In: Chemoprevention of cancer and DNA damage by dietary factors, (S. Knasmüller DMD, I. Johnson and C. Gerhäuser, ed). Weinheim, Germany:Wiley-VCH Verlag GmbH & Co. KGaA.

Slaga TJ, Fischer SM, Weeks CE, Klein-Szanto AJ. 1980. Multistage chemical carcinogenesis in mouse skin. Current problems in dermatology 10:193-218.

Smith MT. 1996. The mechanism of benzene-induced leukemia: A hypothesis and speculations on the causes of leukemia. Environ Health Perspect 104 Suppl 6:1219-1225.

Smith MT, Skibola CF, Allan JM, Morgan GJ. 2004. Causal models of leukaemia and lymphoma. IARC scientific publications:373-392.

Environ Health Perspect DOI: 10.1289/ehp.1509912
Advance Publication: Not Copyedited

Suman S, Sharma PK, Rai G, Mishra S, Arora D, Gupta P, et al. 2015. Current perspectives of molecular pathways involved in chronic inflammation-mediated breast cancer. Biochemical and biophysical research communications.

Thomas RS, Philbert MA, Auerbach SS, Wetmore BA, Devito MJ, Cote I, et al. 2013. Incorporating new technologies into toxicity testing and risk assessment: Moving from 21st century vision to a data-driven framework. Toxicological sciences : an official journal of the Society of Toxicology 136:4-18.

Trinchieri G. 2012. Cancer and inflammation: An old intuition with rapidly evolving new concepts. Annu Rev Immunol 30:677-706.

Wallace BD, Redinbo MR. 2013. Xenobiotic-sensing nuclear receptors involved in drug metabolism: A structural perspective. Drug metabolism reviews 45:79-100.

Yugawa T, Kiyono T. 2009. Molecular mechanisms of cervical carcinogenesis by high-risk human papillomaviruses: Novel functions of e6 and e7 oncoproteins. Reviews in medical virology 19:97-113.

Environ Health Perspect DOI: 10.1289/ehp.1509912
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Table 1. Key characteristics of carcinogens.

Characteristic	Examples of relevant evidence
1. Is Electrophilic or Can Be Metabolically Activated	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone, etc), formation of DNA and protein adducts.
2. Is Genotoxic	DNA damage (DNA strand breaks, DNA-protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei).
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)
4. Induces Epigenetic Alterations	DNA methylation, histone modification, microRNA expression
5. Induces Oxidative Stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Is Immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor in/activation (e.g., ER, PPAR, AhR) or modulation of exogenous ligands (including hormones)
9. Causes Immortalization	Inhibition of senescence, cell transformation
10. Alters cell proliferation, cell death or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis

Any of the 10 characteristics in this table could interact with any other (e.g. oxidative stress, DNA damage and chronic inflammation, which when combined provides stronger evidence for a cancer mechanism than would oxidative stress alone).

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Figure Legends

Figure 1: Literature flow diagram, illustrating the systematic identification and categorization process for benzene mechanistic studies. Using appropriate MeSH terms and key words, targeted literature searches were conducted for the 10 key characteristics using online tools available from the HAWC Project (https://hawcproject.org/). Section 4 refers to the location of the discussion of mechanistic data within the IARC Monograph structure (http://monographs.iarc.fr/ENG/Preamble/currentb4studiesother0706.php). All inclusion categories were expanded to document the number of studies attributed to each, down to the individual key characteristic level, which were expanded to illustrate human information when >100 total studies were identified. Less frequently encountered key characteristic categories (grey circles) were left unexpanded for clarity. Human refers to both humans exposed in vivo and human cells exposed in vitro.

Figure 2: An overview of how benzene induces 8 of the key characteristics in a probable mechanism of carcinogenicity. A full review of these mechanistic data is given in (McHale et al. 2012), from which this Figure was adapted.

Figure 3: An overview of how polychlorinated biphenyls (PCBs) may induce 7 key characteristics in their carcinogenicity (Lauby-Secretan et al. 2013). Highly chlorinated PCBs act as ligands for the aryl hydrocarbon receptor (AhR) and other receptors activating a large number of genes in a tissue- and cell-specific manner that can lead to cell proliferation, apoptosis and other effects that influence cancer risk. Less chlorinated PCBs can be activated to electrophilic metabolites, such as arene oxides and quinones, which can cause genotoxic effects and induce oxidative stress. Receptor binding to CAR and AhR (a key characteristic) leads

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xenobiotic metabolism induction (not a key characteristic, brown not blue box) that in turn leads to genotoxicity and other key characteristics.

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Figure 1

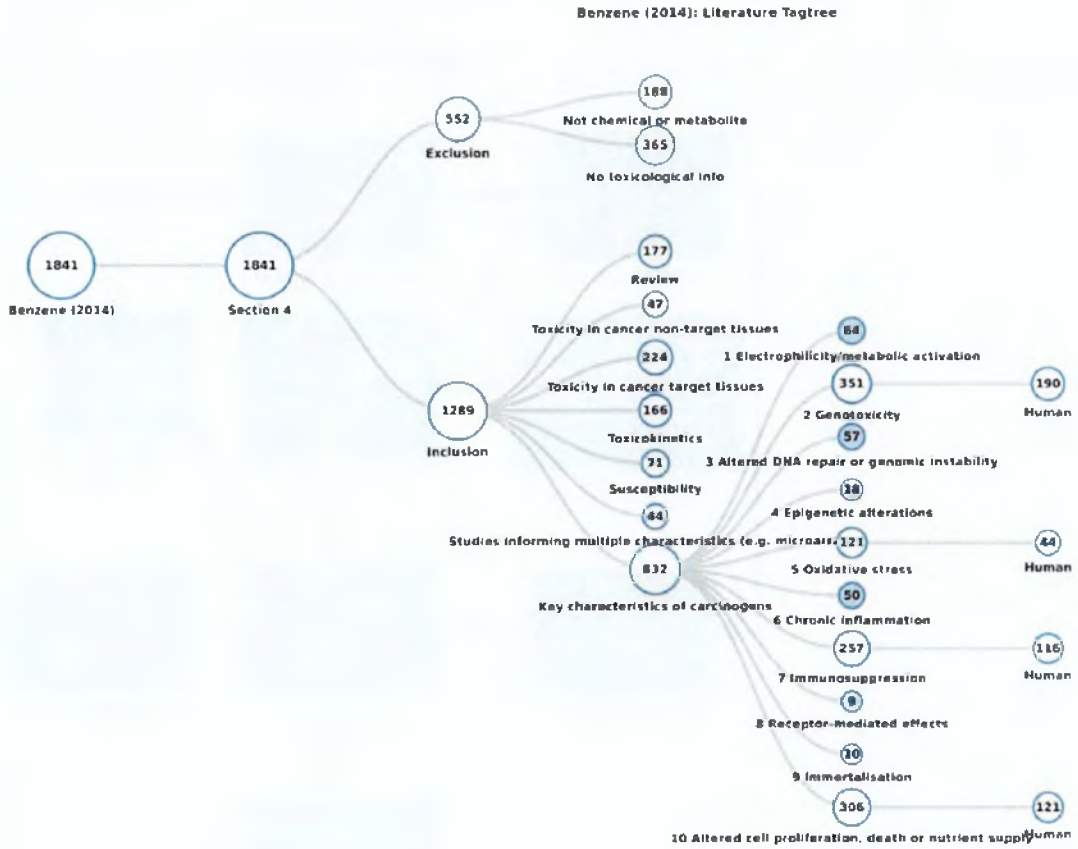

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Figure 2

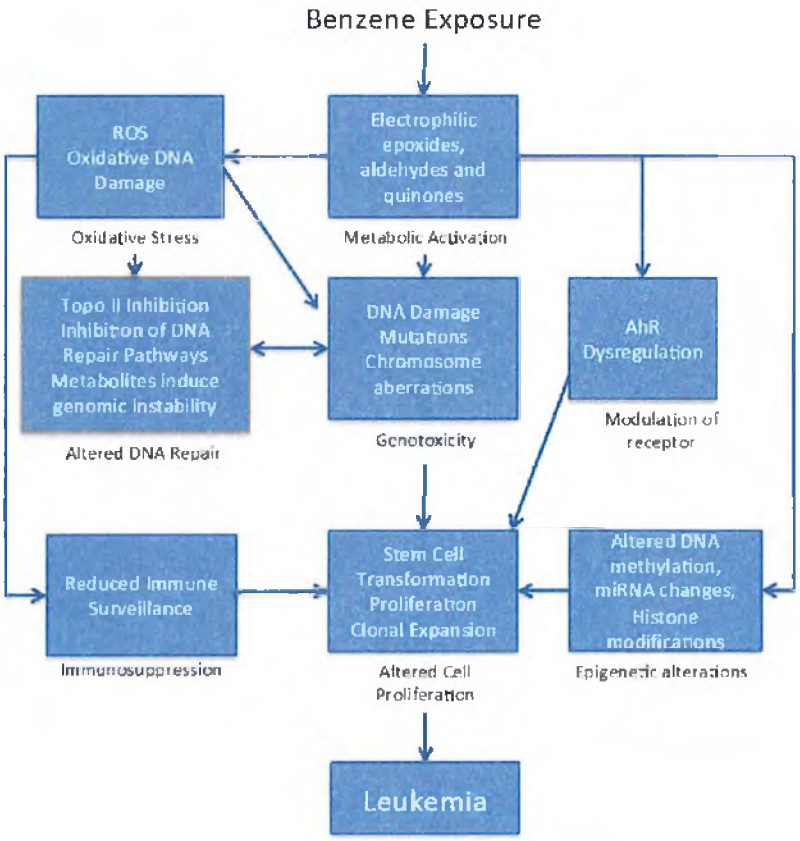

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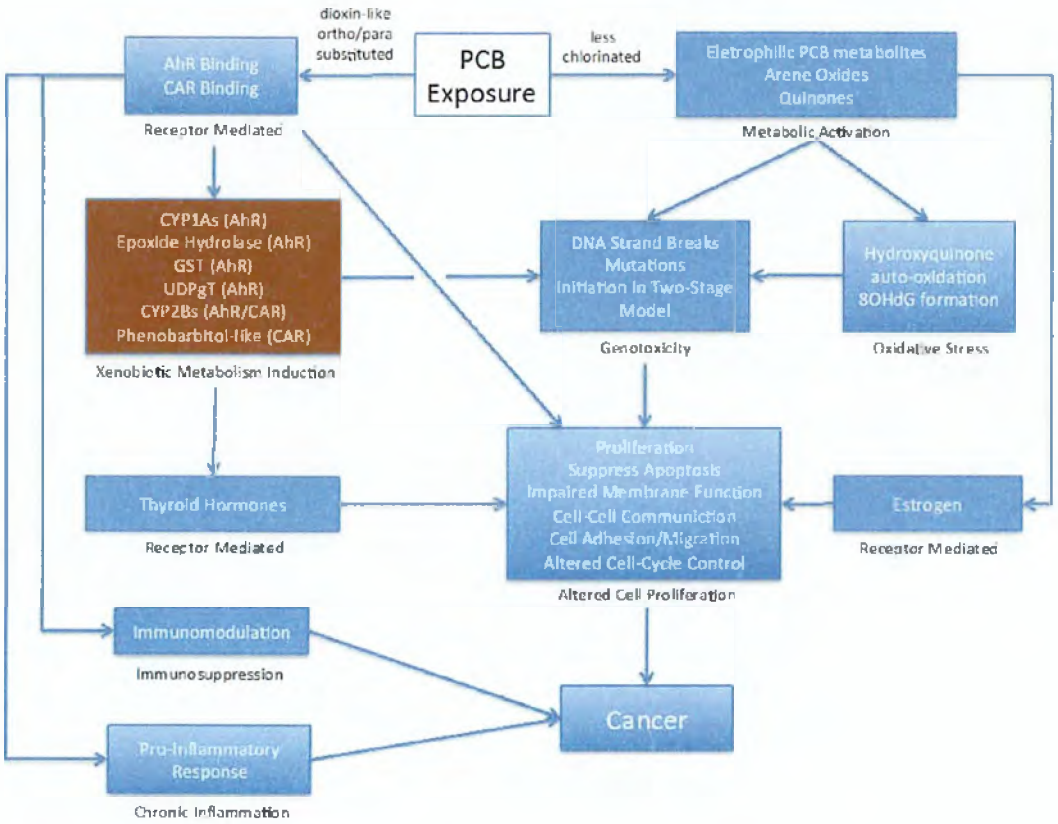
Figure 3



GLYPHOSATE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 1071-83-6 (acid); also relevant:
38641-94-0 (glyphosate-isopropylamine salt)
40465-66-5 (monoammonium salt)
69254-40-6 (diammonium salt)
34494-03-6 (glyphosate-sodium)
81591-81-3 (glyphosate-trimesium)

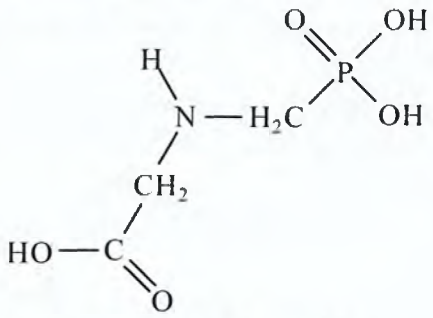
Chem. Abstr. Serv. Name: *N*-(phosphonomethyl)glycine

Preferred IUPAC Name: *N*-(phosphonomethyl)glycine

Synonyms: Gliphosate; glyphosate; glyphosate hydrochloride; glyphosate [calcium, copper (2+), dilithium, disodium, magnesium, monoammonium, monopotassium, monosodium, sodium, or zinc] salt

Trade names: Glyphosate products have been sold worldwide under numerous trade names, including: Abundit Extra; Credit; Xtreme; Glifonox; Glyphogan; Ground-Up; Rodeo; Roundup; Touchdown; Tragli; Wipe Out; Yerbimat (Farm Chemicals International, 2015).

1.1.2 Structural and molecular formulae and relative molecular mass



Molecular formula: $C_3H_8NO_5P$
Relative molecular mass: 169.07

Additional information on chemical structure is also available in the PubChem Compound database (NCBI, 2015).

1.1.3 Chemical and physical properties of the pure substance

Description: Glyphosate acid is a colourless, odourless, crystalline solid. It is formulated as a salt consisting of the deprotonated acid of glyphosate and a cation (isopropylamine, ammonium, or sodium), with more than one salt in some formulations.

Solubility: The acid is of medium solubility at 11.6 g/L in water (at 25 °C) and insoluble in common organic solvents such as acetone, ethanol, and xylene; the alkali-metal and

amine salts are readily soluble in water (Tomlin, 2000).

Volatility: Vapour pressure, 1.31×10^{-2} mPa at 25 °C (negligible) (Tomlin, 2000).

Stability: Glyphosate is stable to hydrolysis in the range of pH 3 to pH 9, and relatively stable to photodegradation (Tomlin, 2000). Glyphosate is not readily hydrolysed or oxidized in the field (Rueppel *et al.* 1977). It decomposes on heating, producing toxic fumes that include nitrogen oxides and phosphorus oxides (IPCS, 2005).

Reactivity: Attacks iron and galvanized steel (IPCS, 2005).

Octanol/water partition coefficient (P): log P, < –3.2 (pH 2–5, 20 °C) (OECD method 107) (Tomlin, 2000).

Henry's law: $< 2.1 \times 10^{-7}$ Pa m^3 mol^{-1} (Tomlin, 2000).

Conversion factor: Assuming normal temperature (25 °C) and pressure (101 kPa), mg/m^3 = 6.92 × ppm.

1.1.4 Technical products and impurities

Glyphosate is formulated as an isopropylamine, ammonium, or sodium salt in water-soluble concentrates and water-soluble granules. The relevant impurities in glyphosate technical concentrates are formaldehyde (maximum, 1.3 g/kg), *N*-nitrosoglyphosate (maximum, 1 mg/kg), and *N*-nitroso-*N*-phosphonomethylglycine (FAO, 2000). Surfactants and sulfuric and phosphoric acids may be added to formulations of glyphosate, with type and concentration differing by formulation (IPCS, 1994).

1.2 Production and use

1.2.1 Production

(a) Manufacturing processes

Glyphosate was first synthesized in 1950 as a potential pharmaceutical compound, but its herbicidal activity was not discovered until it was re-synthesized and tested in 1970 (Székács & Darvas, 2012). The isopropylamine, sodium, and ammonium salts were introduced in 1974, and the trimesium (trimethylsulfonium) salt was introduced in Spain in 1989. The original patent protection expired outside the USA in 1991, and within the USA in 2000. Thereafter, production expanded to other major agrochemical manufacturers in the USA, Europe, Australia, and elsewhere (including large-scale production in China), but the leading preparation producer remained in the USA (Székács & Darvas, 2012).

There are two dominant families of commercial production of glyphosate, the "alkyl ester" pathways, predominant in China, and the "iminodiacetic acid" pathways, with iminodiacetic acid produced from iminodiacetonitrile (produced from hydrogen cyanide), diethanol amine, or chloroacetic acid (Dill *et al.*, 2010; Tian *et al.*, 2012).

To increase the solubility of technical-grade glyphosate acid in water, it is formulated as its isopropylamine, monoammonium, potassium, sodium, or trimesium salts. Most common is the isopropylamine salt, which is formulated as a liquid concentrate (active ingredient, 5.0–62%), ready-to-use liquid (active ingredient, 0.5–20%), pressurized liquid (active ingredient, 0.75–0.96%), solid (active ingredient, 76–94%), or pellet/tablet (active ingredient, 60–83%) (EPA, 1993a).

There are reportedly more than 750 products containing glyphosate for sale in the USA alone (NPIC, 2010). Formulated products contain various non-ionic surfactants, most notably polyethyloxylated tallowamine (POEA), to

facilitate uptake by plants (Székács & Darvas, 2012). Formulations might contain other active ingredients, such as simasine, 2,4-dichlorophenoxyacetic acid (2,4-D), or 4-chloro-2-methylphenoxyacetic acid (IPCS, 1996), with herbicide resistance driving demand for new herbicide formulations containing multiple active ingredients (Freedonia, 2012).

(b) Production volume

Glyphosate is reported to be manufactured by at least 91 producers in 20 countries, including 53 in China, 9 in India, 5 in the USA, and others in Australia, Canada, Cyprus, Egypt, Germany, Guatemala, Hungary, Israel, Malaysia, Mexico, Singapore, Spain, Taiwan (China), Thailand, Turkey, the United Kingdom, and Venezuela (Farm Chemicals International, 2015). Glyphosate was registered in over 130 countries as of 2010 and is probably the most heavily used herbicide in the world, with an annual global production volume estimated at approximately 600 000 tonnes in 2008, rising to about 650 000 tonnes in 2011, and to 720 000 tonnes in 2012 (Dill *et al.*, 2010; CCM International, 2011; Hilton, 2012; Transparency Market Research, 2014).

Production and use of glyphosate have risen dramatically due to the expiry of patent protection (see above), with increased promotion of non-till agriculture, and with the introduction in 1996 of genetically modified glyphosate-tolerant crop varieties (Székács & Darvas, 2012). In the USA alone, more than 80 000 tonnes of glyphosate were used in 2007 (rising from less than 4000 tonnes in 1987) (EPA, 1997, 2011). This rapid growth rate was also observed in Asia, which accounted for 30% of world demand for glyphosate in 2012 (Transparency Market Research, 2014). In India, production increased from 308 tonnes in 2003–2004, to 2100 tonnes in 2007–2008 (Ministry of Chemicals & Fertilizers, 2008). China currently produces more than 40% of the global supply of glyphosate, exports almost 35% of the global supply (Hilton, 2012), and reportedly has sufficient production capacity to satisfy total global demand (Yin, 2011).

1.2.2 Uses

Glyphosate is a broad-spectrum, post-emergent, non-selective, systemic herbicide, which effectively kills or suppresses all plant types, including grasses, perennials, vines, shrubs, and trees. When applied at lower rates, glyphosate is a plant-growth regulator and desiccant. It has agricultural and non-agricultural uses throughout the world.

(a) Agriculture

Glyphosate is effective against more than 100 annual broadleaf weed and grass species, and more than 60 perennial weed species (Dill *et al.*, 2010). Application rates are about 1.5–2 kg/ha for pre-harvest, post-planting, and pre-emergence use; about 4.3 kg/ha as a directed spray in vines, orchards, pastures, forestry, and industrial weed control; and about 2 kg/ha as an aquatic herbicide (Tomlin, 2000). Common application methods include broadcast, aerial, spot, and directed spray applications (EPA, 1993a).

Due to its broad-spectrum activity, the use of glyphosate in agriculture was formerly limited to post-harvest treatments and weed control between established rows of tree, nut, and vine crops. Widespread adoption of no-till and conservation-till practices (which require chemical weed control while reducing soil erosion and labour and fuel costs) and the introduction of transgenic crop varieties engineered to be resistant to glyphosate have transformed glyphosate to a post-emergent, selective herbicide for use on annual crops (Duke & Powles, 2009; Dill *et al.* 2010). Glyphosate-resistant transgenic varieties have been widely adopted for the production of corn, cotton, canola, and soybean (Duke & Powles, 2009). Production of such crops accounted for 45% of worldwide demand for glyphosate in 2012 (Transparency Market Research, 2014). However, in Europe,

where the planting of genetically modified crops has been largely restricted, post-harvest treatment is still the most common application of glyphosate (Glyphosate Task Force, 2014). Intense and continuous use of glyphosate has led to the emergence of resistant weeds that may reduce its effectiveness (Duke & Powles, 2009).

(b) Residential use

Glyphosate is widely used for household weed control throughout the world. In the USA, glyphosate was consistently ranked as the second most commonly used pesticide (after 2,4-D) in the home and garden market sector between 2001 and 2007, with an annual use of 2000–4000 tonnes (EPA, 2011).

(c) Other uses

Glyphosate was initially used to control perennial weeds on ditch banks and roadsides and under power lines (Dill *et al.*, 2010). It is also used to control invasive species in aquatic or wetland systems (Tu *et al.*, 2001). Approximately 1–2% of total glyphosate use in the USA is in forest management (Mance, 2012).

Glyphosate has been used in a large-scale aerial herbicide-spraying programme begun in 2000 to reduce the production of cocaine in Colombia (Lubick, 2009), and of marijuana in Mexico and South America (Székács & Darvas, 2012).

(d) Regulation

Glyphosate has been registered for use in at least 130 countries (Dill *et al.*, 2010). In the USA, all uses are eligible for registration on the basis of a finding that glyphosate "does not pose unreasonable risks or adverse effects to humans or the environment" (EPA, 1993a). A review conducted in 2001 in connection with the registration process in the European Union reached similar conclusions regarding animal and human safety, although the protection of groundwater during non-crop use was identified as requiring particular attention in the short term (European Commission, 2002).

Nevertheless, as worldwide rates of adoption of herbicide-resistant crops and of glyphosate use have risen in recent years (Duke & Powles, 2009), restriction of glyphosate use has been enacted or proposed in several countries, although documented actions are few. In 2013, the Legislative Assembly of El Salvador voted a ban on the use of pesticides containing glyphosate (República de El Salvador, 2013). Sri Lanka is reported to have instituted a partial ban based on an increasing number of cases of chronic kidney disease among agricultural workers, but the ban was lifted after 2 months (ColomboPage, 2014). The reasons for such actions have included the development of resistance among weed species, as well as health concerns.

No limits for occupational exposure were identified by the Working Group.

1.3 Measurement and analysis

Several methods exist for the measurement of glyphosate and its major metabolite aminomethyl phosphonic acid (AMPA) in various media, including air, water, urine, and serum (Table 1.1). The methods largely involve derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) to reach sufficient retention in chromatographic columns (Kuang *et al.*, 2011; Botero-Coy *et al.*, 2013). Chromatographic techniques that do not require derivatization and enzyme-linked immunosorbent assays (ELISA) are under development (Sanchis *et al.*, 2012).

Table 1.1 Methods for the analysis of glyphosate

Sample matrix	Assay procedure	Limit of detection	Reference
Water	HPLC/MS (with online solid-phase extraction)	0.08 µg/L	Lee *et al.* (2001)
	ELISA	0.05 µg/L	Abraxis (2005)
	LC-LC-FD	0.02 µg/L	Hidalgo *et al.* (2004)
	Post HPLC column derivatization and FD	6.0 µg/L	EPA (1992)
	UV visible spectrophotometer (at 435 ng)	1.1 µg/L	Jan *et al.* (2009)
Soil	LC–MS/MS with triple quadrupole	0.02 mg/kg	Botero-Coy *et al.* (2013)
Dust	GC-MS-MID	0.0007 mg/kg	Curwin *et al.* (2005)
Air	HPLC/MS with online solid-phase extraction	0.01 ng/m³	Chang *et al.* (2011)
Fruits and vegetables	HILIC/WAX with ESI-MS/MS	1.2 µg/kg	Chen *et al.* (2013)
Field crops (rice, maize and soybean)	LC–ESI-MS/MS	0.007–0.12 mg/kg	Botero-Coy *et al.* (2013b)
Plant vegetation	HPLC with single polymeric amino column	0.3 mg/kg	Nedelkoska & Low (2004)
Serum	LC–MS/MS	0.03 µg/mL 0.02 µg/mL (aminomethylphosphonic acid) 0.01 µg/mL (3-methylphosphinicopropionic acid)	Yoshioka *et al.* (2011)
Urine	HPLC with post-column reaction and FD	1 µg/L	Acquavella *et al.* (2004)
	ELISA	0.9 µg/L	Curwin *et al.* (2007)

ELISA, enzyme-linked immunosorbent assay; ESI-MS/MS, electrospray tandem mass spectrometry; FD, fluorescence detection; GC-MS-MID, gas chromatography-mass spectrometry in multiple ion detection mode; HILIC/WAX, hydrophilic interaction/weak anion-exchange liquid chromatography; HPLC/MS, high-performance liquid chromatography with mass spectrometry; HPLC, high-performance liquid chromatography; LC-ESI-MS/MS, liquid chromatography-electrospray–tandem mass spectrometry; LC-LC, coupled-column liquid chromatography; LC–MS/MS, liquid chromatography–tandem mass spectrometry

1.4 Occurrence and exposure

1.4.1 Exposure

(a) Occupational exposure

Studies related to occupational exposure to glyphosate have included farmers and tree nursery workers in the USA, forestry workers in Canada and Finland, and municipal weed-control workers in the United Kingdom (Centre de Toxicologie du Québec, 1988; Jauhiainen *et al.*, 1991; Lavy *et al.*, 1992; Acquavella *et al.*, 2004; Johnson *et al.*, 2005). Para-occupational exposures to glyphosate have also been measured in farming families (Acquavella *et al.*, 2004; Curwin *et al.*, 2007). These studies are summarized in Table 1.2.

(b) Community exposure

Glyphosate can be found in soil, air, surface water, and groundwater (EPA, 1993a). Once in the environment, glyphosate is adsorbed to soil and is broken down by soil microbes to AMPA (Borggaard & Gimsing, 2008). In surface water, glyphosate is not readily broken down by water or sunlight (EPA, 1993a). Despite extensive worldwide use, there are relatively few studies

Table 1.2 Occupational and para-occupational exposure to glyphosate

Industry, country, year	Job/process	Results	Comments/additional data	Reference
Forestry				
Canada, 1986		Arithmetic mean of air glyphosate concentrations:	Air concentrations of glyphosate were measured at the work sites of one crew (five workers) during ground spraying 268 urine samples were collected from 40 workers; glyphosate concentration was above the LOD (15 µg/L) in 14%	Centre de Toxicologie du Québec (1988)
	Signaller	Morning, 0.63 µg/m³ Afternoon, 2.25 µg/m³		
	Operator	Morning, 1.43 µg/m³ Afternoon, 6.49 µg/m³		
	Overseer	Morning, 0.84 µg/m³ Afternoon, 2.41 µg/m³		
	Mixer	Morning, 5.15 µg/m³ Afternoon, 5.48 µg/m³		
Finland, year NR	Workers performing silvicultural clearing (n = 5)	Range of air glyphosate concentrations, < 1.25–15.7 µg/m³ (mean, NR)	Clearing work was done with brush saws equipped with pressurized herbicide sprayers Air samples were taken from the workers' breathing zone (number of samples, NR) Urine samples were collected during the afternoons of the working week (number, NR) Glyphosate concentrations in urine were below the LOD (10 µg/L)	Jauhiainen *et al.* (1991)
USA, year NR	Workers in two tree nurseries (n = 14)	In dermal sampling, 1 of 78 dislodgeable residue samples were positive for glyphosate The body portions receiving the highest exposure were ankles and thighs	Dermal exposure was assessed with gauze patches attached to the clothing and hand rinsing Analysis of daily urine samples repeated over 12 weeks was negative for glyphosate	Lavy *et al.* (1992)
Weed control				
United Kingdom, year NR	Municipal weed control workers (n = 18)	Median, 16 mg/m³ in 85% of 21 personal air samples for workers spraying with mechanized all-terrain vehicle Median, 0.12 mg/m³ in 33% of 12 personal air samples collected from workers with backpack with lance applications	[The Working Group noted that the reported air concentrations were substantially higher than in other studies, but was unable to confirm whether the data were for glyphosate or total spray fluid] Dermal exposure was also measured, but reported as total spray fluid, rather than glyphosate	Johnson *et al.* (2005)

Table 1.2 (continued)

Industry, country, year	Job/process	Results	Comments/additional data	Reference
Farming				
USA, 2001	Occupational and para-occupational exposure of 24 farm families (24 fathers, 24 mothers and 65 children). Comparison group: 25 non-farm families (23 fathers, 24 mothers and 51 children)	Geometric mean (range) of glyphosate concentrations in urine: Non-farm fathers, 1.4 µg/L (0.13–5.4) Farm fathers, 1.9 µg/L (0.02–18) Non-farm mothers, 1.2 µg/L (0.06–5.0) Farm mothers, 1.5 µg/L (0.10–11) Non-farm children, 2.7 µg/L (0.10–9.4) Farm children, 2.0 µg/L (0.02–18)	Frequency of glyphosate detection ranged from 66% to 88% of samples (observed concentrations below the LOD were not censored). Detection frequency and geometric mean concentration were not significantly different between farm and non-farm families (observed concentrations below the LOD were not censored)	Curwin *et al.* (2007)
USA, year NR	Occupational and para-occupational exposures of 48 farmers, their spouses, and 79 children	Geometric mean (range) of glyphosate concentration in urine on day of application: Farmers, 3.2 µg/L (< 1 to 233 µg/L) Spouses, NR (< 1 to 3 µg/L) Children, NR (< 1 to 29 µg/L)	24-hour composite urine samples for each family member the day before, the day of, and for 3 days after a glyphosate application. Glyphosate was detected in 60% of farmers' samples, 4% of spouses' samples and 12% of children's samples the day of spraying and in 27% of farmers' samples, 2% of spouses' samples and 5% of children's samples 3 days after	Acquavella *et al.* (2004)

LOD, limit of detection; ND, not detected; NR, not reported

on the environmental occurrence of glyphosate (Kolpin *et al.*, 2006).

(i) Air

Very few studies of glyphosate in air were available to the Working Group. Air and rainwater samples were collected during two growing seasons in agricultural areas in Indiana, Mississippi, and Iowa, USA (Chang *et al.*, 2011). The frequency of glyphosate detection ranged from 60% to 100% in air and rain samples, and concentrations ranged from < 0.01 to 9.1 ng/m^3 in air samples and from < 0.1 to 2.5 µg/L in rainwater samples. Atmospheric deposition was measured at three sites in Alberta, Canada. Rainfall and particulate matter were collected as total deposition at 7-day intervals throughout the growing season. Glyphosate deposition rates ranged from < 0.01 to 1.51 µg/m^2 per day (Humphries *et al.*, 2005).

No data were available to the Working Group regarding glyphosate concentrations in indoor air.

(ii) Water

Glyphosate in the soil can leach into groundwater, although the rate of leaching is believed to be low (Borggaard & Gimsing, 2008; Simonsen *et al.*, 2008). It can also reach surface waters by direct emission, atmospheric deposition, and by adsorption to soil particles suspended in runoff water (EPA, 1993a; Humphries *et al.*, 2005). Table 1.3 summarizes data on concentrations of glyphosate or AMPA in surface water and groundwater.

(iii) Residues in food and dietary intake

Glyphosate residues have been measured in cereals, fruits, and vegetables (Table 1.4). Residues were detected in 0.04% of 74 305 samples of fruits, vegetables, and cereals tested from 27 member states of the European Union, and from Norway, and Iceland in 2007 (EFSA, 2009). In cereals, residues were detected in 50% of samples tested in Denmark in 1998–1999, and in 9.5% of samples tested from member states of the European Union, and from Norway and Iceland in 2007 (Granby & Vahl, 2001; EFSA, 2009). In the United Kingdom, food sampling for glyphosate residues has concentrated mainly on cereals, including bread and flour. Glyphosate has been detected regularly and usually below the reporting limit (Pesticide Residues Committee, 2007, 2008, 2009, 2010). Six out of eight samples of tofu made from Brazilian soy contained glyphosate, with the highest level registered being 1.1 mg/kg (Pesticide Residues Committee, 2007).

(iv) Household exposure

In a survey of 246 California households, 14% were found to possess at least one product containing glyphosate (Guha *et al.*, 2013).

(v) Biological markers

Glyphosate concentrations in urine were analysed in urban populations in Europe, and in a rural population living near areas sprayed for drug eradication in Colombia (MLHB, 2013; Varona *et al.*, 2009). Glyphosate concentrations in Colombia were considerably higher than in Europe, with means of 7.6 ng/L and 0.02 µg/L, respectively (Table 1.5). In a study in Canada, glyphosate concentrations in serum ranged from undetectable to 93.6 ng/mL in non-pregnant women (n = 39), and were undetectable in serum of pregnant women (n = 30) and fetal cord serum (Aris & Leblanc, 2011).

1.4.2 Exposure assessment

Exposure assessment methods in epidemiological studies on glyphosate and cancer are discussed in Section 2.0 of the *Monograph* on Malathion, in the present volume.

Table 1.3 Concentration of glyphosate and AMPA in water

Country, year of sampling	Number of samples/setting	Results	Comments/additional data	Reference
USA, 2002	51 streams/agricultural areas (154 samples)	Maximum glyphosate concentration, 5.1 µg/L Maximum AMPA concentration, 3.67 µg/L	The samples were taken following pre- and post-emergence application and during harvest season Glyphosate detected in 36% of samples; AMPA detected in 69% of samples	Battaglin *et al.*, (2005)
USA, 2002	10 wastewater treatment plants and two reference streams (40 samples)	Glyphosate, range ≤ 0.1–2 µg/L AMPA, range ≤ 0.1–4 µg/L	AMPA was detected more frequently (67.5%) than glyphosate (17.5%)	Kolpin *et al.* (2006)
Canada, 2002	3 wetlands and 10 agricultural streams (74 samples)	Range, < 0.02–6.08 µg/L	Glyphosate was detected in most of the wetlands and streams (22% of samples)	Humphries *et al.* (2005)
Colombia, year NR	5 areas near crops and coca eradication (24 samples)	Maximum concentration, 30.1 µg/L (minimum and mean, NR)	Glyphosate detected in 8% of samples (MDL, 25 µg/L)	Solomon *et al.*, (2007)
Denmark, 2010–2012	4 agricultural sites (450 samples)	Range, < 0.1–31.0 µg/L	Glyphosate detected in 23% of samples; AMPA detected in 25% of samples	Brüch *et al.* (2013)

AMPA, aminomethylphosphonic acid; MDL, method detection limit; NR, data not reported

Table 1.4 Concentrations of glyphosate in food

Country, year	Type of food	Results	Comments/additional data	Reference
Denmark, 1998, 1999	Cereals	> 50% of samples had detectable residues Means: 0.08 mg/kg in 1999 and 0.11 mg/kg in 1998	49 samples of the 1998 harvest 46 samples of the 1999 harvest	Granby & Vahl (2001)
27 European Union member states, Norway and Iceland, 2007	350 different food commodities	0.04% of 2302 fruit, vegetable and cereal samples 9.5% of 409 cereal samples	74 305 total samples	EFSA (2009)
Australia, 2006	Composite sample of foods consumed in 24 hours	75% of samples had detectable residues Mean, 0.08 mg/kg Range, < 0.005 to 0.5 mg/kg	20 total samples from 43 pregnant women	McQueen *et al.* (2012)

Table 1.5 Concentrations of glyphosate and AMPA in urine and serum in the general population

Country, period	Subjects	Results	Comments/additional data	Reference
Urine				
18 European countries, 2013	162 individuals	Arithmetic mean of glyphosate concentration: 0.21 µg/L (maximum, 1.56 µg/L) Arithmetic mean of AMPA concentration: 0.19 µg/L (maximum, 2.63 µg/L)	44% of samples had quantifiable levels of glyphosate and 36% had quantifiable levels of AMPA	MLHB (2013)
Colombia, 2005–2006	112 residents of areas sprayed for drug eradication	Arithmetic mean (range) of glyphosate concentration: 7.6 µg/L (ND–130 µg/L) Arithmetic mean (range) of AMPA concentration: 1.6 µg/L (ND–56 µg/L)	40% of samples had detectable levels of glyphosate and 4% had detectable levels of AMPA (LODs, 0.5 and 1.0 µg/L, respectively) Urinary glyphosate was associated with use in agriculture	Varona *et al.* (2009)
Serum				
Canada, NR	30 pregnant women and 39 non-pregnant women	ND in serum of pregnant women or cord serum; Arithmetic mean, 73.6 µg/L, (range, ND–93.6 µg/L) in non-pregnant women	No subject had worked or lived with a spouse working in contact with pesticides LOD, 15 µg/L	Aris & Leblanc (2011)

AMPA, aminomethylphosphonic acid; LOD, limit of detection; ND, not detected; NR, not reported

2. Cancer in Humans

2.0 General discussion of epidemiological studies

A general discussion of the epidemiological studies on agents considered in Volume 112 of the *IARC Monographs* is presented in Section 2.0 of the *Monograph* on Malathion.

2.1 Cohort studies

See Table 2.1

The Agricultural Health Study (AHS), a large prospective cohort study conducted in Iowa and North Carolina in the USA, is the only cohort study to date to have published findings on exposure to glyphosate and the risk of cancer at many different sites (Alavanja *et al.*, 1996; NIH, 2015) (see Section 2.0 of the *Monograph* on Malathion, in the present volume, for a detailed description of this study).

The enrolment questionnaire from the AHS sought information on the use of 50 pesticides (ever or never exposure), crops grown and livestock raised, personal protective equipment used, pesticide application methods used, other agricultural activities and exposures, nonfarm occupational exposures, and several lifestyle, medical, and dietary variables. The duration (years) and frequency (days per year) of use was investigated for 22 of the 50 pesticides in the enrolment questionnaire. [Blair *et al.* (2011) assessed the possible impact of misclassification of occupational pesticide exposure on relative risks, demonstrating that nondifferential exposure misclassification biases relative risk estimates towards the null in the AHS and tends to decrease the study power.]

The first report of cancer incidence associated with pesticide use in the AHS cohort considered cancer of the prostate (Alavanja *et al.*, 2003). Risk estimates for exposure to glyphosate were not presented, but no significant exposure–response association with cancer of the prostate was found. In an updated analysis of the AHS (1993 to 2001), De Roos *et al.* (2005a) (see below) also found no association between exposure to glyphosate and cancer of the prostate (relative risk, RR, 1.1; 95% CI, 0.9–1.3) and no exposure–response trend (*P* value for trend = 0.69).

De Roos *et al.* (2005a) also evaluated associations between exposure to glyphosate and the incidence of cancer at several other sites. The prevalence of ever-use of glyphosate was 75.5% (> 97% of users were men). In this analysis, exposure to glyphosate was defined as: (a) ever personally mixed or applied products containing glyphosate; (b) cumulative lifetime days of use, or "cumulative exposure days" (years of use × days/year); and (c) intensity-weighted cumulative exposure days (years of use × days/year × estimated intensity level). Poisson regression was used to estimate exposure–response relations between exposure to glyphosate and incidence of all cancers combined, and incidence of 12 cancer types: lung, melanoma, multiple myeloma, and non-Hodgkin lymphoma (see Table 2.1) as well as oral cavity, colon, rectum, pancreas, kidney, bladder, prostate, and leukaemia (results not tabulated). Exposure to glyphosate was not associated with all cancers combined (RR, 1.0; 95% CI, 0.9–1.2; 2088 cases). For multiple myeloma, the relative risk was 1.1 (95% CI, 0.5–2.4; 32 cases) when adjusted for age, but was 2.6 (95% CI, 0.7–9.4) when adjusted for multiple confounders (age, smoking, other pesticides, alcohol consumption, family history of cancer, and education); in analyses by cumulative exposure-days and intensity-weighted exposure-days, the relative risks were around 2.0 in the highest tertiles. Furthermore, the association between multiple myeloma and exposure to glyphosate only appeared within the subgroup for which complete data were available on all the covariates; even without any adjustment, the risk of multiple myeloma associated with glyphosate use was increased by twofold among the smaller subgroup with available covariate data

Table 2.1 Cohort studies of cancer and exposure to glyphosate

Reference, study location, enrolment period/follow-up, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
De Roos *et al.* (2005a) Iowa and North Carolina, USA 1993–2001	54 315 (after exclusions, from a total cohort of 57 311) licensed pesticide applicators Exposure assessment method: questionnaire; semi-quantitative assessment from self-administered questionnaire	Lung	Ever use	147	0.9 (0.6–1.3)	Age, smoking, other pesticides, alcohol consumption, family history of cancer, education	AHS Cancer sites investigated: lung, melanoma, multiple myeloma and NHL (results tabulated) as well as oral cavity, colon, rectum, pancreas, kidney, bladder, prostate and leukaemia (results not tabulated) [Strengths: large cohort; specific assessment of glyphosate; semiquantitative exposure assessment. Limitations: risk estimates based on self-reported exposure; limited to licensed applicators; potential exposure to multiple pesticides]
			Cumulative exposure days:				
			1–20	40	1 (ref.)		
			21–56	26	0.9 (0.5–1.5)		
			57–2678	26	0.7 (0.4–1.2)		
			Trend-test *P* value: 0.21				
		Melanoma	Ever use	75	1.6 (0.8–3)		
			1–20	23	1 (ref.)		
			21–56	20	1.2 (0.7–2.3)		
			57–2678	14	0.9 (0.5–1.8)		
			Trend-test *P* value: 0.77				
		Multiple myeloma	Ever use	32	1.1 (0.5–2.4)	Age only (results in this row only)	
			Ever use	32	2.6 (0.7–9.4)		
			1–20	8	1 (ref.)		
			21–56	5	1.1 (0.4–3.5)		
			Trend-test *P* value: 0.27				
		NHL	Ever use	92	1.1 (0.7–1.9)		
			1–20	29	1 (ref.)		
			21–56	15	0.7 (0.4–1.4)		
			57–2678	17	0.9 (0.5–1.6)		
			Trend-test *P* value: 0.73				

Table 2.1 (continued)

Reference, study location, enrolment period/follow-up, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Flower *et al.* (2004) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up, 1975–1998	21 375; children (aged < 19 years) of licensed pesticide applicators in Iowa (n = 17 357) and North Carolina (n = 4018) Exposure assessment method: questionnaire	Childhood cancer	Maternal use of glyphosate (ever) Paternal use of glyphosate (prenatal)	13 6	0.61 (0.32–1.16) 0.84 (0.35–2.34)	Child's age at enrolment	AHS Glyphosate results relate to the Iowa participants only [Strengths: Large cohort; specific assessment of glyphosate. Limitations: based on self-reported exposure; potential exposure to multiple pesticides; limited power for glyphosate exposure]
Engel *et al.* (2005) Iowa and North Carolina, USA Enrolment, 1993–1997 follow-up to 2000	30 454 wives of licensed pesticide applicators with no history of breast cancer at enrolment Exposure assessment method: questionnaire	Breast	Direct exposure to glyphosate Husband's use of glyphosate	82 109	0.9 (0.7–1.1) 1.3 (0.8–1.9)	Age, race, state	AHS [Strengths: large cohort; specific assessment of glyphosate. Limitations: based on self-reported exposure; limited to licensed applicators; potential exposure to multiple pesticides]
Lee *et al.* (2007) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up to 2002	56 813 licensed pesticide applicators Exposure assessment method: questionnaire	Colorectum Colon Rectum	Exposed to glyphosate Exposed to glyphosate Exposed to glyphosate	225 151 74	1.2 (0.9–1.6)	Age, smoking, state, total days of any pesticide application	AHS [Strengths: large cohort. Limitations: based on self-reported exposure, limited to licensed applicators, potential

Table 2.1 (continued)

Reference, study location, enrolment period/follow-up, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Andreotti *et al.* (2009) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up to 2004 Nested case–control study	Cases: 93 (response rate, NR); identified from population-based state-cancer registries. Incident cases diagnosed between enrolment and 31 December 2004 (> 9 years follow-up) included in the analysis. Participants with any type of prevalent cancer at enrolment were excluded. Vital status was obtained from the state death registries and the National Death Index. Participants who left North Carolina or Iowa were not subsequently followed for cancer occurrence. Controls: 82 503 (response rate, NR); cancer-free participants enrolled in the cohort Exposure assessment method: questionnaire providing detailed pesticide use, demographic and lifestyle information. Ever-use of 24 pesticides and intensity-weighted lifetime days [(lifetime exposure days) × (exposure intensity score)] of 13 pesticides was assessed	Pancreas (C25.0–C25.9)	Ever exposure to glyphosate Low (< 185 days) High (≥ 185 days) Trend-test *P* value: 0.85	55 29 19	1.1 (0.6–1.7)	Age, smoking, diabetes	AHS [Strengths: large cohort. Limitations: based on self-reported exposure; limited to licensed applicators; potential exposure to multiple pesticides]

AHS, Agricultural Health Study; NHL, non-Hodgkin lymphoma; NR, not reported

(De Roos *et al.*, 2005b). [The study had limited power for the analysis of multiple myeloma; there were missing data on covariates when multiple adjustments were done, limiting the interpretation of the findings.] A re-analysis of these data conducted by Sorahan (2015) confirmed that the excess risk of multiple myeloma was present only in the subset with no missing information (of 22 cases in the restricted data set). In a subsequent cross-sectional analysis of 678 male participants from the same cohort, Landgren *et al.* (2009) did not find an association between exposure to glyphosate and risk of monoclonal gammopathy of undetermined significance (MGUS), a premalignant plasma disorder that often precedes multiple myeloma (odds ratio, OR, 0.5; 95% CI, 0.2–1.0; 27 exposed cases).

Flower *et al.* (2004) reported the results of the analyses of risk of childhood cancer associated with pesticide application by parents in the AHS. The analyses for glyphosate were conducted among 17 357 children of Iowa pesticide applicators from the AHS. Parents provided data via questionnaires (1993–1997) and the cancer follow-up (retrospectively and prospectively) was done through the state cancer registries. Fifty incident childhood cancers were identified (1975–1998; age, 0–19 years). For all the children of the pesticide applicators, risk was increased for all childhood cancers combined, for all lymphomas combined, and for Hodgkin lymphoma, compared with the general population. The odds ratio for use of glyphosate and risk of childhood cancer was 0.61 (95% CI, 0.32–1.16; 13 exposed cases) for maternal use and 0.84 (95% CI, 0.35–2.34; 6 exposed cases) for paternal use. [The Working Group noted that this analysis had limited power to study a rare disease such as childhood cancer.]

Engel *et al.* (2005) reported on incidence of cancer of the breast among farmers' wives in the AHS cohort, which included 30 454 women with no history of cancer of the breast before enrolment in 1993–1997. Information on pesticide use and other factors was obtained at enrolment by self-administered questionnaire from the women and their husbands. A total of 309 incident cases of cancer of the breast were identified until 2000. There was no difference in incidence of cancer of the breast for women who reported ever applying pesticides compared with the general population. The relative risk for cancer of the breast among women who had personally used glyphosate was 0.9 (95% CI, 0.7–1.1; 82 cases) and 1.3 (95% CI, 0.8–1.9; 109 cases) among women who never used pesticides but whose husband had used glyphosate. [No information on duration of glyphosate use by the husband was presented.] Results for glyphosate were not further stratified by menopausal status.

Lee *et al.* (2007) investigated the relationship between exposure to agricultural pesticides and incidence of cancer of the colorectum in the AHS. A total of 56 813 pesticide applicators with no prior history of cancer of the colorectum were included in this analysis, and 305 incident cancers of the colorectum (colon, 212; rectum, 93) were diagnosed during the study period, 1993–2002. Most of the 50 pesticides studied were not associated with risk of cancer of the colorectum, and the relative risks with exposure to glyphosate were 1.2 (95% CI, 0.9–1.6), 1.0 (95% CI, 0.7–1.5), and 1.6 (95% CI, 0.9–2.9) for cancers of the colorectum, colon, and rectum, respectively.

Andreotti *et al.* (2009) examined associations between the use of pesticides and cancer of the pancreas using a case–control analysis nested in the AHS. This analysis included 93 incident cases of cancer of the pancreas (64 applicators, 29 spouses) and 82 503 cancer-free controls who completed the enrolment questionnaire. Ever-use of 24 pesticides and intensity-weighted lifetime days [(lifetime exposure days) × (exposure intensity score)] of 13 pesticides were assessed. Risk estimates were calculated controlling for age, smoking, and diabetes. The odds ratio for ever- versus never-exposure to glyphosate was

1.1 (95% CI, 0.6–1.7; 55 exposed cases), while the odds ratio for the highest category of level of intensity-weighted lifetime days was 1.2 (95% CI, 0.6–2.6; 19 exposed cases).

Dennis *et al.* (2010) reported that exposure to glyphosate was not associated with cutaneous melanoma within the AHS. [The authors did not report a risk estimate.]

2.2 Case–control studies on non-Hodgkin lymphoma, multiple myeloma, and leukaemia

2.2.1 Non-Hodgkin lymphoma

See Table 2.2

(a) Case–control studies in the midwest USA

Cantor *et al.* (1992) conducted a case–control study of incident non-Hodgkin lymphoma (NHL) among males in Iowa and Minnesota, USA (see the *Monograph* on Malathion, Section 2.0, for a detailed description of this study). A total of 622 white men and 1245 population-based controls were interviewed in person. The association with farming occupation and specific agricultural exposures were evaluated. When compared with non-farmers, the odds ratios for NHL were 1.2 (95% CI, 1.0–1.5) for men who had ever farmed, and 1.1 (95% CI, 0.7–1.9; 26 exposed cases; adjusted for vital status, age, state, cigarette smoking status, family history of lymphohaematopoietic cancer, high-risk occupations, and high-risk exposures) for ever handling glyphosate. [There was low power to assess the risk of NHL associated with exposure to glyphosate. There was no adjustment for other pesticides. These data were included in the pooled analysis by De Roos *et al.* (2003).]

Brown *et al.* (1993) reported the results of a study to evaluate the association between multiple myeloma and agricultural risk factors in the midwest USA (see the *Monograph* on Malathion, Section 2.0, for a detailed description of this study). A population-based case–control study of 173 white men with multiple myeloma and 650 controls was conducted in Iowa, USA, an area with a large farming population. A non-significantly elevated risk of multiple myeloma was seen among farmers compared with never-farmers. The odds ratio related to exposure to glyphosate was 1.7 (95% CI, 0.8–3.6; 11 exposed cases). [This study had limited power to assess the association between multiple myeloma and exposure to glyphosate. Multiple myeloma is now considered to be a subtype of NHL.]

De Roos *et al.* (2003) used pooled data from three case–control studies of NHL conducted in the 1980s in Nebraska (Zahm *et al.*, 1990), Kansas (Hoar *et al.*, 1986), and in Iowa and Minnesota (Cantor *et al.*, 1992) (see the *Monograph* on Malathion, Section 2.0, for a detailed description of these studies) to examine pesticide exposures in farming as risk factors for NHL in men. The study population included 870 cases and 2569 controls; 650 cases and 1933 controls were included for the analysis of 47 pesticides controlling for potential confounding by other pesticides. Both logistic regression and hierarchical regression (adjusted estimates were based on prior distributions for the pesticide effects, which provides more conservative estimates than logistic regression) were used in data analysis, and all models were essentially adjusted for age, study site, and other pesticides. Reported use of glyphosate as well as several individual pesticides was associated with increased incidence of NHL. Based on 36 cases exposed, the odds ratios for the association between exposure to glyphosate and NHL were 2.1 (95% CI, 1.1–4.0) in the logistic regression analyses and 1.6 (95% CI, 0.9–2.8) in the hierarchical regression analysis. [The numbers of cases and controls were lower than those in the pooled analysis by Waddell *et al.* (2001) because only subjects with no missing data on pesticides were included. The strengths of this study when compared with other studies are that it was large,

Table 2.2 Case–control studies of leukaemia and lymphoma and exposure to glyphosate

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
USA							
Brown *et al.* (1990) Iowa and Minnesota, USA 1981–1983	Cases: 578 (340 living, 238 deceased) (response rate, 86%); cancer registry or hospital records Controls: 1245 (820 living, 425 deceased) (response rate, 77–79%); random-digit dialling for those aged < 65 years and Medicare for those aged ≥ 65 years Exposure assessment method: questionnaire	Leukaemia	Any glyphosate	15	0.9 (0.5–1.6)	Age, vital status, state, tobacco use, family history lymphopoietic cancer, high-risk occupations, high risk exposures	[Strengths: large population based study in a farming area. Limitations: not controlled for exposure to other pesticides. Limited power for glyphosate exposure]
Cantor *et al.* (1992) Iowa and Minnesota, USA 1980–1982	Cases: 622 (response rate, 89.0%); Iowa health registry records and Minnesota hospital and pathology records Controls: 1245 (response rate, 76–79%); population-based; no cancer of the lympho-haematopoietic system; frequency-matched to cases by age (5-year group), vital status, state. Random-digit dialling (aged < 65 years); Medicare records (aged ≥ 65 years); state death certificate files (deceased subjects) Exposure assessment method: questionnaire; in-person interview	NHL	Ever handled glyphosate	26	1.1 (0.7–1.9)	Age, vital status, state, smoking status, family history lymphopoietic cancer, high-risk occupations, high-risk exposures	Data subsequentially pooled in De Roos *et al.* (2003); white men only [Strengths: large population-based study in farming areas. Limitations: not controlled for exposure to other pesticides. Limited power for glyphosate exposure]

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Brown *et al.* (1993) Iowa, USA 1981–1984	Cases: 173 (response rate, 84%); Iowa health registry Controls: 650 (response rate, 78%); Random-digit dialling (aged < 65 years) and Medicare (aged > 65 years) Exposure assessment method: questionnaire	Multiple myeloma	Any glyphosate	11	1.7 (0.8–3.6)	Age, vital status	[Strengths: population-based study. Areas with high prevalence of farming. Limitations: limited power for glyphosate exposure]
De Roos *et al.* (2003) Nebraska, Iowa, Minnesota, Kansas, USA 1979–1986	Cases: 650 (response rate, 74.7%); cancer registries and hospital records Controls: 1933 (response rate, 75.2%); random-digit dialling, Medicare, state mortality files Exposure assessment method: questionnaire; interview (direct or next-of-kin)	NHL	Any glyphosate exposure	36	2.1 (1.1–4)	Age, study area, other pesticides	Both logistic regression and hierarchical regression were used in data analysis, the latter providing more conservative estimates [Strengths: increased power when compared with other studies, population-based, and conducted in farming areas. Advanced analytical methods to account for multiple exposures] Included participants from Cantor *et al.* (1992), Zahm *et al.* (1990), Hoar *et al.* (1986), and Brown *et al.* (1990)

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Lee *et al.* (2004a) Iowa, Minnesota and Nebraska, USA 1980–1986	Cases: 872 (response rate, NR); diagnosed with NHL from 1980 to 1986 Controls: 2381 (response rate, NR); frequency-matched controls Exposure assessment method: questionnaire; information on use of pesticides and history of asthma was based on interviews	NHL	Exposed to glyphosate – non-asthmatics Exposed to glyphosate – asthmatics	53 6	1.4 (0.98–2.1) 1.2 (0.4–3.3)	Age, vital status, state	177 participants (45 NHL cases, 132 controls) reported having been told by their doctor that they had asthma
Canada							
McDuffie *et al.* (2001) Canada 1991–1994	Cases: 517 (response rate, 67.1%), from cancer registries and hospitals Controls: 1506 (response rate, 48%); random sample from health insurance and voting records Exposure assessment method: questionnaire, some administered by telephone, some by post	NHL	Exposed to glyphosate Unexposed > 0 and ≤ 2 days > 2 days	51 464 28 23	1.2 (0.83–1.74) 1 1.0 (0.63–1.57) 2.12 (1.2–3.73)	Age, province of residence	Cross-Canada study [Strengths: large population based study. Limitations: no quantitative exposure data. Exposure assessment by questionnaire. Relatively low participation]

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Karunanayake *et al.* (2012) Six provinces in Canada (Quebec, Ontario, Manitoba, Saskatchewan, Alberta, and British Columbia) 1991–1994	Incident cases: 316 (response rate, 68.4%); men aged ≥ 19 years; ascertained from provincial cancer registries, except in Quebec (hospital ascertainment) Controls: 1506 (response rate, 48%); matched by age ± 2 years to be comparable with the age distribution of the entire case group (HL, NHL, MM, and STS) within each province of residence. Potential controls (men aged ≥ 19 years) selected at random within age constraints from the provincial health insurance records (Alberta, Saskatchewan, Manitoba, Quebec), computerized telephone listings (Ontario), or voters' lists (British Columbia) Exposure assessment method: questionnaire; stage 1 used a self-administered postal questionnaire; and in stage 2 detailed pesticide exposure information was collected by telephone interview	HL (ICDO2 included nodular sclerosis (M9656/3; M9663/3; M9664/3; M9665/3; M9666/3; M9667/3), lymphocytic predominance (M9651/3; M9657/3; M9658/3; M9659/3), mixed cellularity (M9652/3), lymphocytic depletion (M9653/3; M9654/3), miscellaneous (other M9650-M9669 codes for HL)	Glyphosate-based formulation	38	1.14 (0.74–1.76)	Age group, province of residence	Cross Canada study Based on the statistical analysis of pilot study data, it was decided that the most efficient definition of pesticide exposure was a cumulative exposure ≥ 10 hours/year to any combination of pesticides. This discriminated (a) between incidental, bystander, and environmental exposure vs more intensive exposure, and (b) between cases and controls [Strengths: large study. Limitations: low response rates]
			Glyphosate-based formulation	38	0.99 (0.62–1.56)	Age group, province of residence, medical history	

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kachuri *et al.* (2013) Six Canadian provinces (British Columbia, Alberta, Saskatchewan, Manitoba, Ontario and Quebec) 1991–1994	Cases: 342 (response rate, 58%); men aged ≥ 19 years diagnosed between 1991 and 1994 were ascertained from provincial cancer registries except in Quebec, where ascertained from hospitals Controls: 1357 (response rate, 48%); men aged ≥ 19 years selected randomly using provincial health insurance records, random digit dialling, or voters' lists, frequency-matched to cases by age (±2 years) and province of residence Exposure assessment method: questionnaire	Multiple myeloma	Glyphosate use	32	1.19 (0.76–1.87)	Age, province of residence, use of a proxy respondent, smoking status, medical variables, family history of cancer	Cross-Canada study [Strengths: population-based case–control study. Limitations: relatively low response rates]
			Use of glyphosate (> 0 and ≤ 2 days per year)	15	0.72 (0.39–1.32)		
			Use of glyphosate (> 2 days per year)	12	2.04 (0.98–4.23)		
Sweden							
Nordström *et al.* (1998) Sweden 1987–1992	Cases: 111 (response rate, 91%); 121 HCL cases in men identified from Swedish cancer registry Controls: 400 (response rate, 83%); 484 (four controls/case) matched for age and county; national population registry Exposure assessment method: questionnaire; considered exposed if minimum exposure of 1 working day (8 h) and an induction period of at least 1 year	HCL	Exposed to glyphosate	4	3.1 (0.8–12)	Age	Overlaps with Hardell *et al.* (2002). HCL is a subtype of NHL [Strengths: population-based case–control study. Limitations: Limited power. There was no adjustment for other exposures]

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Hardell & Eriksson (1999) Northern and middle Sweden 1987–1990	Cases: 404 (192 deceased) (response rate, 91%); regional cancer registries Controls: 741 (response rate, 84%); live controls matched for age and county were recruited from the national population registry, and deceased cases matched for age and year of death were identified from the national registry for causes of death Exposure assessment method: questionnaire	NHL (ICD-9 200 and 202)	Ever glyphosate – univariate Ever glyphosate – multivariate	4 NR	2.3 (0.4–13) 5.8 (0.6–54)	Not specified in the multivariable analysis	Overlaps with Hardell *et al.* (2002) [Strengths: population-based study. Limitations: few subjects were exposed to glyphosate and the study had limited power. Analyses were "multivariate" but covariates were not specified]
Hardell *et al.* (2002) Sweden; four Northern counties and three counties in mid Sweden 1987–1992	Cases: 515 (response rate, 91% in both studies); Swedish cancer registry Controls: 1141 (response rates, 84% and 83%%); national population registry Exposure assessment method: questionnaire	NHL and HCL	Ever glyphosate exposure (univariate) Ever glyphosate exposure (multivariate)	8 8	3.04 (1.08–8.5) 1.85 (0.55–6.2)	Age, county, study site, vital status, other pesticides in the multivariate analysis	Overlaps with Nordström *et al.* (1998) and Hardell & Eriksson (1999). [Strengths: large population-based study. Limitations: limited power for glyphosate exposure]

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Eriksson *et al.* (2008) Sweden. Four health service areas (Lund, Linkoping, Orebro and Umea) 1999–2002	Cases: 910 (response rate, 91%); incident NHL cases were enrolled from university hospitals Controls: 1016 (response rate, 92%); national population registry Exposure assessment method: questionnaire	NHL	Any glyphosate	29	2.02 (1.1–3.71)	Age, sex, year of enrolment	[Strengths: population-based case-control. Limitations: limited power for glyphosate] * Exposure to other pesticides (e.g. MPCA) controlled in the analysis
			Any glyphosate*	29	1.51 (0.77–2.94)		
			≤ 10 days per year use	12	1.69 (0.7–4.07)		
			> 10 days per year use	17	2.36 (1.04–5.37)		
		NHL	1–10 yrs	NR	1.11 (0.24–5.08)		
			> 10 yrs	NR	2.26 (1.16–4.4)		
		B-cell lymphoma	Exposure to glyphosate	NR	1.87 (0.998–3.51)		
		Lymphocytic lymphoma/B-CLL	Exposure to glyphosate	NR	3.35 (1.42–7.89)		
		Diffuse large B-cell lymphoma	Exposure to glyphosate	NR	1.22 (0.44–3.35)		
		Follicular, grade I–III	Exposure to glyphosate	NR	1.89 (0.62–5.79)		
		Other specified B-cell lymphoma	Exposure to glyphosate	NR	1.63 (0.53–4.96)		
		Unspecified B-cell lymphoma	Exposure to glyphosate	NR	1.47 (0.33–6.61)		
		T-cell lymphoma	Exposure to glyphosate	NR	2.29 (0.51–10.4)		
		Unspecified NHL	Exposure to glyphosate	NR	5.63 (1.44–22)		

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Other studies in Europe							
Orsi *et al.* (2009) France 2000–2004	Cases: 491 (response rate, 95.7%); cases (244 NHL; 87 HL; 104 LPSs; 56 MM) were recruited from main hospitals of the French cities of Brest, Caen, Nantes, Lille, Toulouse and Bordeaux, aged 20–75 years; ALL cases excluded Controls: 456 (response rate, 91.2%); matched on age and sex, recruited in the same hospitals as the cases, mainly in orthopaedic and rheumatological departments and residing in the hospital's catchment area Exposure assessment method: questionnaire	NHL	Any glyphosate exposure	12	1.0 (0.5–2.2)	Age, centre, socioeconomic category (blue/ white collar)	[Limitations: limited power for glyphosate]
		HL	Any exposure to glyphosate	6	1.7 (0.6–5)		
		LPS	Any exposure to glyphosate	4	0.6 (0.2–2.1)		
		MM	Any exposure to glyphosate	5	2.4 (0.8–7.3)		
		All lymphoid neoplasms	Any exposure to glyphosate	27	1.2 (0.6–2.1)		
		NHL, diffuse large cell lymphoma	Occupational use of glyphosate	5	1.0 (0.3–2.7)		
		NHL, follicular lymphoma	Occupational exposure to glyphosate	3	1.4 (0.4–5.2)		
		LPS/CLL	Occupational exposure to glyphosate	2	0.4 (0.1–1.8)		
		LPS/HCL	Occupational exposure to glyphosate	2	1.8 (0.3–9.3)		

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Cocco *et al.* (2013) Czech Republic, France, Germany, Italy, Ireland and Spain 1998–2004	Cases: 2348 (response rate, 88%); cases were all consecutive adult patients first diagnosed with lymphoma during the study period, resident in the referral area of the participating centres Controls: 2462 (response rate, 81% hospital; 52% population); controls from Germany and Italy were randomly selected by sampling from the general population and matched to cases on sex, 5-year age-group, and residence area. The rest of the centres used matched hospital controls, excluding diagnoses of cancer, infectious diseases and immunodeficiency diseases Exposure assessment method: questionnaire; support of a crop-exposure matrix to supplement the available information, industrial hygienists and occupational experts in each participating centre reviewed the general questionnaires and job modules to assess exposure to pesticides	B-cell lymphoma	Occupational exposure to glyphosate	4	3.1 (0.6–17.1)	Age, sex, education, centre	EPILYMPH case-control study in six European countries

ALL, acute lymphocytic leukaemia; B-CLL, chronic lymphocytic leukaemia; CLL, chronic lymphocytic leukaemia; HCL, hairy cell leukaemia; HL, Hodgkin lymphoma; LPS, lymphoproliferative syndrome; MCPA, 2-methyl-4-chlorophenoxyacetic acid; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; NR, not reported; ref., reference; STS, soft tissue sarcoma

population-based, and conducted in farming areas. Potential confounding from multiple exposures was accounted for in the analysis.]

Using the data set of the pooled population-based case–control studies in Iowa, Minnesota, and Nebraska, USA, Lee *et al.* (2004a) investigated whether asthma acts as an effect modifier of the association between pesticide exposure and NHL. The study included 872 cases diagnosed with NHL from 1980 to 1986 and 2381 frequency-matched controls. Information on use of pesticides and history of asthma was based on interviews. A total of 177 subjects (45 cases, 132 controls) reported having been told by their doctor that they had asthma. Subjects with a history of asthma had a non-significantly lower risk of NHL than non-asthmatics, and there was no main effect of pesticide exposure. In general, asthmatics tended to have larger odds ratios associated with exposure to pesticides than non-asthmatics. There was no indication of effect modification: the odds ratio associated with glyphosate use was 1.4 (95% CI, 0.98–2.1; 53 exposed cases) among non-asthmatics and 1.2 (95% CI, 0.4–3.3; 6 exposed cases) for asthmatics, when compared with non-asthmatic non-exposed farmers). [This analysis overlapped with that of De Roos *et al.* (2003).]

(b) The cross-Canada case–control study

McDuffie *et al.* (2001) studied the associations between exposure to specific pesticides and NHL in a multicentre population-based study with 517 cases and 1506 controls among men of six Canadian provinces (see the *Monograph* on Malathion, Section 2.0, for a detailed description of this study). Odds ratios of 1.26 (95% CI, 0.87–1.80; 51 exposed cases; adjusted for age and province) and 1.20 (95% CI, 0.83–1.74, adjusted for age, province, high-risk exposures) were observed for exposure to glyphosate. In an analysis by frequency of exposure to glyphosate, participants with > 2 days of exposure per year had an odds ratio of 2.12 (95% CI, 1.20–3.73, 23 exposed cases) compared with those with some, but ≤ 2 days of exposure. [The study was large, but had relatively low participation rates.]

Kachuri *et al.* (2013) investigated the association between lifetime use of pesticides and multiple myeloma in a population-based case–control study among men in six Canadian provinces between 1991 and 1994 (see the *Monograph* on Malathion, Section 2.0, for a detailed description of this study). Data from 342 cases of multiple myeloma and 1357 controls were obtained for ever-use of pesticides, number of pesticides used, and days per year of pesticide use. The odds ratios were adjusted for age, province of residence, type of respondent, smoking and medical history. The odds ratio for ever-use of glyphosate was 1.19 (95% CI, 0.76–1.87; 32 cases). When the analysis was conducted by level of exposure, no association was found for light users (≤ 2 days per year) of glyphosate (OR, 0.72; 95% CI, 0.39–1.32; 15 exposed cases) while the odds ratio in heavier users (> 2 days per year) was 2.04 (95% CI, 0.98–4.23; 12 exposed cases). [The study had relatively low response rates. Multiple myeloma is now considered a subtype of NHL.]

(c) Case–control studies in Sweden

Nordström *et al.* (1998) conducted a population case–control study in Sweden on hairy cell leukaemia (considered to be a subgroup of NHL). The study included 121 cases in men and 484 controls matched for age and sex. An age-adjusted odds ratio of 3.1 (95% CI, 0.8–12; 4 exposed cases) was observed for exposure to glyphosate. [This study had limited power to detect an effect, and there was no adjustment for other exposures.]

Hardell & Eriksson (1999) reported the results of a population-based case–control study on the incidence of NHL in men associated with pesticide exposure in four northern counties in Sweden. Exposure data was collected by questionnaire (also supplemented by telephone interviews) from 404 cases (192 deceased) and 741

controls (matched by age, sex, county, and vital status). Increased risks of NHL were found for subjects exposed to herbicides and fungicides. The odds ratio for ever-use of glyphosate was 2.3 (95% CI, 0.4–13; 4 exposed cases) in a univariate analysis, and 5.8 (95% CI, 0.6–54) in a multivariable analysis. [The exposure frequency was low for glyphosate, and the study had limited power to detect an effect. The variables included in the multivariate analysis were not specified. This study may have overlapped partially with those of Hardell *et al.* (2002).]

Hardell *et al.* (2002) conducted a pooled analysis of two case–control studies, one on NHL (already reported in Hardell & Eriksson, 1999) and another on hairy cell leukaemia, a subtype of NHL (already reported by Nordström *et al.*, 1998). The pooled analysis of NHL and hairy cell leukaemia was based on 515 cases and 1141 controls. Increased risk was found for exposure to glyphosate (OR, 3.04; 95% CI, 1.08–8.52; 8 exposed cases) in the univariate analysis, but the odds ratio decreased to 1.85 (95% CI, 0.55–6.20) when study, study area, and vital status were considered in a multivariate analysis. [The exposure frequency was low for glyphosate and the study had limited power. This study partially overlapped with those of Hardell & Eriksson (1999) and Nordström *et al.* (1998).]

Eriksson *et al.* (2008) reported the results of a population based case–control study of exposure to pesticides as a risk factor for NHL. Men and women aged 18–74 years living in Sweden were included from 1 December 1999 to 30 April 2002. Incident cases of NHL were enrolled from university hospitals in Lund, Linköping, Örebro, and Umeå. Controls (matched by age and sex) were selected from the national population registry. Exposure to different agents was assessed by questionnaire. In total, 910 (91%) cases and 1016 (92%) controls participated. Multivariable models included agents with statistically significant increased odds ratios (MCPA, 2-methyl-4-chlorophenoxyacetic acid), or with an odds ratio of > 1.50 and at least 10 exposed subjects (2,4,5-T and/or 2,4-D; mercurial seed dressing, arsenic, creosote, tar), age, sex, year of diagnosis or enrolment. The odds ratio for exposure to glyphosate was 2.02 (95% CI, 1.10–3.71) in a univariate analysis, and 1.51 (95% CI, 0.77–2.94) in a multivariable analysis. When exposure for more than 10 days per year was considered, the odds ratio was 2.36 (95% CI, 1.04–5.37). With a latency period of > 10 years, the odds ratio was 2.26 (95% CI, 1.16–4.40). The associations with exposure to glyphosate were reported also for lymphoma subtypes, and elevated odds ratios were reported for most of the cancer forms, including B-cell lymphoma (OR, 1.87; 95% CI, 0.998–3.51) and the subcategory of small lymphocytic lymphoma/chronic lymphocytic leukaemia (OR, 3.35; 95% CI, 1.42–7.89; [not adjusted for other pesticides]). [This was a large study; there was possible confounding from use of other pesticides including MCPA, but this was considered in the analysis.]

(d) Other case–control studies in Europe

Orsi *et al.* (2009) reported the results of a hospital-based case–control study conducted in six centres in France between 2000 and 2004. Incident cases with a diagnosis of lymphoid neoplasm aged 20–75 years and controls of the same age and sex as the cases were recruited in the same hospital, mainly in the orthopaedic and rheumatological departments during the same period. [The Working Group noted that the age of case eligibility was given in the publication as 20–75 years in the materials and methods section, but as 18–75 years in the abstract.] Exposures to pesticides were evaluated through specific interviews and case-by-case expert reviews. The analyses included 491 cases (244 cases of NHL, 87 cases of Hodgkin lymphoma), 104 of lymphoproliferative syndrome, and 56 cases of multiple myeloma), and 456 age- and sex-matched controls. Positive associations between some subtypes and occupational exposure to several pesticides

were noted. The odds ratios associated with any exposure to glyphosate were 1.2 (95% CI, 0.6–2.1; 27 exposed cases) for all lymphoid neoplasms combined, 1.0 (95% CI, 0.5–2.2; 12 exposed cases) for NHL, 0.6 (95% CI, 0.2–2.1; 4 exposed cases) for lymphoproliferative syndrome, 2.4 (95% CI, 0.8–7.3) for multiple myeloma, and 1.7 (95% CI, 0.6–5.0; 6 exposed cases) for Hodgkin lymphoma, after adjusting for age, centre, and socioeconomic category ("blue/white collar").

Cocco *et al.* (2013) reported the results of a pooled analysis of case–control studies conducted in six European countries in 1998–2004 (EPILYMPH, Czech Republic, France, Germany, Ireland, Italy, and Spain) to investigate the role of occupational exposure to specific groups of chemicals in the etiology of lymphoma overall, B-cell lymphoma, and its most prevalent subtypes. A total of 2348 incident cases of lymphoma and 2462 controls were recruited. Controls from Germany and Italy were randomly selected by sampling from the general population, while the rest of the centres used matched hospital controls. Overall, the participation rate was 88% for cases, 81% for hospital controls, and 52% for population controls. An occupational history was collected with farm work-specific questions on type of crop, farm size, pests being treated, type and schedule of pesticide use. In each study centre, industrial hygienists and occupational experts assessed exposure to specific groups of pesticides and individual compounds with the aid of agronomists. [Therefore any exposure misclassification would be non-differential.] Analyses were conducted for lymphoma and the most prevalent lymphoma subtypes adjusting for age, sex, education, and centre. Lymphoma overall, and B-cell lymphoma were not associated with any class of the investigated pesticides, while the risk of chronic lymphocytic leukaemia was elevated among those ever exposed to inorganic and organic pesticides. Only for a few individual agrochemicals was there a sizeable number of study subjects to conduct a meaningful analysis, and the odds ratio for exposure to glyphosate and B-cell lymphoma was 3.1 (95% CI, 0.6–17.1; 4 exposed cases and 2 exposed controls). [The study had a very limited power to assess the effects of glyphosate on risk of NHL.]

2.2.2 Other haematopoietic cancers

Orsi *et al.* (2009) also reported results for Hodgkin lymphoma (see Section 2.2.1).

Karunanayake *et al.* (2012) conducted a case–control study of Hodgkin lymphoma among white men, aged 19 years or older, in six regions of Canada (see the Malathion *Monograph*, Section 2.0, for a detailed description of this study). The analysis included 316 cases and 1506 age-matched (± 2 years) controls. Based on 38 cases exposed to glyphosate, the odds ratios were 1.14 (95% CI, 0.74–1.76) adjusted for age and province, and 0.99 (95% CI, 0.62–1.56) when additionally adjusted for medical history variables.

Brown *et al.* (1990) evaluated exposure to carcinogens in an agricultural setting and the relationship with leukaemia in a population-based case–control interview study in Iowa and Minnesota, USA, including 578 white men with leukaemia and 1245 controls. The exposure assessment was done with a personal interview of the living subjects or the next-of-kin. Farmers had a higher risk of all leukaemias compared with non-farmers, and associations were found for exposure to specific animal insecticides, including the organophosphates crotoxyphos, dichlorvos, famphur, pyrethrins, and methoxychlor. The odds ratio for glyphosate was 0.9 (95% CI, 0.5–1.6; 15 exposed cases; adjusted for vital status, age, state, tobacco use, family history of lymphopoietic cancer, high-risk occupations, and high-risk exposures). [This was a large study in an agricultural setting, but had limited power for studying the effects of glyphosate use.]

2.3 Case–control studies on other cancer sites

2.3.1 Cancer of the oesophagus and stomach

Lee *et al.* (2004b) evaluated the risk of adenocarcinomas of the oesophagus and stomach associated with farming and agricultural pesticide use. The population-based case–control study was conducted in eastern Nebraska, USA. Subjects of both sexes diagnosed with adenocarcinoma of the stomach (n = 170) or oesophagus (n = 137) between 1988 and 1993 were enrolled. Controls (n = 502) were randomly selected from the population registry of the same geographical area. The response rates were 79% for cancer of the stomach, 88% for cancer of the oesophagus, and 83% for controls. Adjusted odds ratios were estimated for use of individual and chemical classes of insecticides and herbicides, with non-farmers as the reference category. No association was found with farming or ever-use of insecticides or herbicides, or with individual pesticides. For ever-use of glyphosate, the odds ratio was 0.8 (95% CI, 0.4–1.4; 12 exposed cases) for cancer of the stomach, and 0.7 (95% CI, 0.3–1.4; 12 exposed cases) for oesophageal cancer. [The study was conducted in a farming area, but the power to detect an effect of glyphosate use was limited.]

2.3.2 Cancer of the brain

Ruder *et al.* (2004) conducted a case–control study on glioma among nonmetropolitan residents of Iowa, Michigan, Minnesota, and Wisconsin in the Upper Midwest Health Study, USA. The study included 457 cases of glioma and 648 population-based controls, all adult men. Exposure assessment was done with interviews of the subject or the relatives. The response rates were 93% and 70% for cases and controls, respectively. No association were found with any of the pesticides assessed, including glyphosate. [Glyphosate use was assessed, but specific results were not presented.]

Carreón *et al.* (2005) evaluated the effects of rural exposures to pesticides on risk of glioma among women aged 18–80 years who were nonmetropolitan residents of Iowa, Michigan, Minnesota, and Wisconsin in the Upper Midwest Health Study, USA. A total of 341 cases of glioma and 528 controls were enrolled. A personal interview was carried out for exposure assessment. The response rates were 90% and 72%, respectively. After adjusting for age, age group, education, and farm residence, no association with glioma was observed for exposure to several pesticide classes or individual pesticides. There was a reduced risk for glyphosate (OR, 0.7; 95% CI, 0.4–1.3; 18 exposed cases). These results were not affected by the exclusion of proxy respondents (43% of cases, 2% of controls).

Lee *et al.* (2005) evaluated the association between farming and agricultural pesticide use and risk of adult glioma in a population-based case–control study in eastern Nebraska, USA. Cases of glioma were in men and women (n = 251) and were compared with population controls from a previous study (n = 498). A telephone interview was conducted for 89% of the cases and 83% of the controls. Adjusted odds ratios for farming and for use of individual and chemical classes of insecticides and herbicides were calculated using non-farmers as the reference category. Among men, ever living or working on a farm and duration of farming were associated with significantly increased risks of glioma, but the positive findings were limited to proxy respondents. Among women, there were no positive associations with farming activities among self or proxy respondents. Some specific pesticide families and individual pesticides were associated with significantly increased risks among male farmers, but most of the positive associations were limited to proxy respondents. There was a non-significant excess risk with glyphosate use for the overall group (OR, 1.5; 95% CI, 0.7–3.1; 17 exposed cases), but there was inconsistency between observations for self-respondents (OR,

0.4; 95% CI, 0.1–1.6) and observations for proxy respondents (OR, 3.1; 95% CI, 1.2–8.2). [The study had limited power to detect an effect of glyphosate use, and the inconsistencies for self and proxy respondents made the results difficult to interpret.]

2.3.3 Soft tissue sarcoma

Pahwa *et al.* (2011) reported the results of the soft tissue sarcoma component of the cross-Canada study in relation to specific pesticides, including 357 cases of soft tissue sarcoma and 1506 population controls from 1991–1994. The fully adjusted odds ratio for glyphosate use was 0.90 (95% CI, 0.58–1.40).

2.3.4 Cancer of the prostate

Band *et al.* (2011) report results of a case–control study including 1516 patients with cancer of the prostate (ascertained by the cancer registry of British Columbia, Canada, for 1983–90) and 4994 age-matched controls with cancers at all other cancer sites excluding lung and unknown primary site. Agricultural exposures were assessed by job-exposure matrix. A total of 60 cases were exposed to glyphosate (adjusted OR, 1.36; 95% CI, 0.83–2.25).

2.3.5 Childhood cancer

Parental exposure to pesticides, including glyphosate, was assessed in a population-based case–control study of childhood leukaemia in Costa Rica (Monge *et al.*, 2007). However, associations of childhood cancer with glyphosate were reported only for an "other pesticides" category that also included paraquat, chlorothalonil, and other chemicals. [Because glyphosate was not specifically assessed, this study was not evaluated by the Working Group.]

2.4. Meta-analyses

Schinasi & Leon (2014) conducted a systematic review and meta-analysis of NHL and occupational exposure to agricultural pesticides, including glyphosate. The meta-analysis for glyphosate included six studies (McDuffie *et al.*, 2001; Hardell *et al.*, 2002; De Roos *et al.*, 2003; 2005a; Eriksson *et al.*, 2008; Orsi *et al.*, 2009) and yielded a meta risk-ratio of 1.5 (95% CI, 1.1–2.0). [The Working Group noted that the most fully adjusted risk estimates from the articles by Hardell *et al.* (2002) and Eriksson *et al.* (2008) were not used in this analysis. After considering the adjusted estimates of the two Swedish studies in the meta-analysis, the Working Group estimated a meta risk-ratio of 1.3 (95% CI, 1.03–1.65), $I^2 = 0\%$, P for heterogeneity 0.589.]

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1

3.1.1 Dietary administration

Groups of 50 male and 50 female CD-1 mice [age not reported] were given diets containing glyphosate (purity, 99.7%) at a concentration of 0, 1000, 5000, or 30 000 ppm, ad libitum, for 24 months. There was no treatment-related effect on body weight in male and female mice at the lowest or intermediate dose. There was a consistent decrease in body weight in the male and female mice at the highest dose compared with controls. Survival in all dose groups was similar to that of controls. There was a positive trend ($P = 0.016$, trend test; see EPA, 1985b) in the incidence of renal tubule adenoma in dosed male mice: 0/49, 0/49, 1/50 (2%), 3/50 (6%). [The Working Group noted that renal tubule adenoma is a rare tumour in CD-1 mice.] No data on tumours of the kidney

Table 3.1 Studies of carcinogenicity with glyphosate in mice

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Mouse, CD-1 (M, F) 24 mo EPA (1985a, b, 1986, 1991a)	Diet containing glyphosate (technical grade; purity, 99.7%) at concentrations of 0, 1000, 5000, or 30 000 ppm, ad libitum, for 24 mo 50 M and 50 F/group [age, NR]	*Males* Renal tubule adenoma: 0/49, 0/49, 1/50 (2%), 3/50 (6%) *Females* No data provided on the kidney Report from the PWG of the EPA (1986): *Males* Renal tubule adenoma: 1/49 (2%), 0/49, 0/50, 1/50 (2%) Renal tubule carcinoma: 0/49, 0/49, 1/50 (2%), 2/50 (4%) Renal tubule adenoma or carcinoma (combined): 1/49 (2%), 0/49, 1/50 (2%), 3/50 (6%)	P for trend = 0.016; see Comments [NS] [P = 0.037; Cochran–Armitage trend test] [P = 0.034; Cochran–Armitage trend test]	No information was provided on renal tubule adenomas in female mice, or on statistical analyses of tumour data EPA recommended that additional renal sections be cut and evaluated from all control and treated male mice. The pathology report for these additional sections (EPA, 1985b) showed the same incidence of renal tubule adenomas as originally reported, with no significant difference in incidence when comparing control and treated groups; however, the test for linear trend in proportions resulted in P = 0.016 EPA (1986) convened a PWG and requested additional pathological and statistical information on kidney tumours observed in male mice treated with glyphosate
Mouse, CD-1 (M, F) 104 wk IMPR (2006)	Diet containing glyphosate (purity, 98.6%) at doses of 0, 100, 300, 1000 mg/kg bw, ad libitum, for 104 wk 50 M and 50 F/group [age, NR]	*Males* Haemangiosarcoma: 0/50, 0/50, 0/50, 4/50 (8%) Histiocytic sarcoma in the lymphoreticular/haemopoietic tissue: 0/50, 2/50 (4%), 0/50, 2/50 (4%) *Females* Haemangiosarcoma: 0/50, 2/50 (4%), 0/50, 1/50 (2%) Histiocytic sarcoma in the lymphoreticular/haemopoietic tissue: 0/50, 3/50 (6%), 3/50 (6%), 1/50 (2%)	[P < 0.001; Cochran–Armitage trend test] NS NS NS	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Mouse, Swiss (M) 32 wk George *et al.* (2010)	Initiation–promotion study Skin application of glyphosate-based formulation (glyphosate, 41%; POEA, ~15%) (referred to as "glyphosate") dissolved in 50% ethanol; DMBA dissolved in 50% ethanol, and TPA dissolved in 50% acetone, used in the groups described below 20 M/group	Skin tumours [called "papillomas" by the authors, following gross examination only]		Short duration of treatment, no solvent controls, and lack of any histopathological evaluation Age at start, NR (mice weighed 12–15 g bw) [The Working Group concluded this was an inadequate study for the evaluation of glyphosate]
	Group I: untreated control (no treatment)	Group I: 0/20		
	Group II: glyphosate only: 25 mg/kg bw topically, 3 × /wk, for 32 wk	Group II: 0/20		
	Group III: single topical application of DMBA, 52 µg/mouse, followed 1 wk later by TPA, 5 µg/mouse, 3 × /wk, for 32 wk	Group III: 20/20*, 7.8 ± 1.1	*$P < 0.05$ vs groups VI and VII	
	Group IV: single topical application of glyphosate, 25 mg/kg bw, followed 1 wk later by TPA, 5 µg/mouse, 3 × /wk, for 32 wk	Group I: 0/20		
	Group V: 3 × /wk topical application of glyphosate, 25 mg/kg bw, for 3 wk, followed 1 wk later by TPA, 5 µg/mouse, 3 × /wk, for 32 wk	Group V: 0/20		
	Group VI: single topical application of DMBA, 52 µg/mouse	Group VI: 0/20		
	Group VII: topical application of TPA, 5 µg/mouse, 3 × /wk, for 32 wk	Group VII: 0/20		
	Group VIII: single topical application of DMBA, 52 µg/mouse, followed 1 wk later by topical treatment with glyphosate, 25 mg/kg bw, 3 × /wk, for 32 wk	Group VIII: 8/20*, 2.8 ± 0.9	*$P < 0.05$ vs group VI	

bw, body weight; DMBA, 7,12-dimethylbenz[*a*]anthracene; EPA, United States Environmental Protection Agency; F, female; M, male; mo, month; NR, not reported; NS, not significant; POEA, polyethoxylated tallowamine; PWG, pathology working group; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate; vs, versus; wk, week; yr, year

were provided for female mice. No other tumour sites were identified (EPA, 1985a). Subsequent to its initial report (EPA, 1985a), the United States Environmental Protection Agency (EPA) recommended that additional renal sections be cut and evaluated from all male mice in the control and treated groups. The pathology report for these additional sections (EPA, 1985b) indicated the same incidence of renal tubule adenoma as originally reported, with no significant increase in incidence between the control group and treated groups by pairwise comparison. However, as already reported above, the test for linear trend in proportions resulted in a significance of $P = 0.016$. The EPA (1986) also requested that a pathology working group (PWG) be convened to evaluate the tumours of the kidney observed in male mice treated with glyphosate, including the additional renal sections. In this second evaluation, the PWG reported that the incidence of adenoma of the renal tubule was 1/49 (2%), 0/49, 0/50, 1/50 (2%) [not statistically significant]; the incidence of carcinoma of the renal tubule was 0/49, 0/49, 1/50 (2%), 2/50 (4%) [$P = 0.037$, trend test for carcinoma]; and the incidence of adenoma or carcinoma (combined) of the renal tubule was 1/49 (2%), 0/49, 1/50 (2%), 3/50 (6%) [$P = 0.034$, trend test for combined]. [The Working Group considered that this second evaluation indicated a significant increase in the incidence of rare tumours, with a dose-related trend, which could be attributed to glyphosate. Chandra & Frith (1994) reported that only 1 out of 725 [0.14%] CD-1 male mice in their historical database had developed renal cell tumours (one carcinoma).]

[The Working Group noted the differences in histopathological diagnosis between pathologists. Proliferative lesions of the renal tubules are typically categorized according to published criteria as hyperplasia, adenoma, or carcinoma. The difference is not trivial, because focal hyperplasia, a potentially preneoplastic lesion, should be carefully differentiated from the regenerative changes of the tubular epithelium. There is a morphological continuum in the development and progression of renal neoplasia. Thus larger masses may exhibit greater heterogeneity in histological growth pattern, and cytologically more pleomorphism and atypia than smaller lesions (Eustis *et al.*, 1994). Of note, a renal tumour confirmed by the PWG after re-evaluation of the original slides (EPA, 1986), had not been seen in the re-sectioned kidney slides (EPA, 1985b). This may be related to the growth of tumour that – in contrast to tumours in other organs – is not spherical but elliptical because of the potential expansion in tubules. In addition, the concept of tubular expansion without compression of adjacent parenchyma may be at the basis of the discrepancy between the first (EPA, 1985a, b) and second evaluation (EPA, 1986).]

In another study reported to the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), groups of 50 male and 50 female CD-1 mice [age at start not reported] were given diets containing glyphosate (purity, 98.6%) at a concentration that was adjusted weekly for the first 13 weeks and every 4 weeks thereafter to give doses of 0, 100, 300, or 1000 mg/kg bw, ad libitum, for 104 weeks (JMPR, 2006). There was no treatment-related effect on body weight or survival in any of the dosed groups. There was an increase in the incidence of haemangiosarcoma in males – 0/50, 0/50, 0/50, 4/50 (8%) [$P < 0.001$, Cochran–Armitage trend test], and in females – 0/50, 2/50 (4%), 0/50, 1/50 (2%) [not statistically significant], and an increase in the incidence of histiocytic sarcoma in the lymphoreticular/haemopoietic tissue in males – 0/50, 2/50 (4%), 0/50, 2/50 (4%), and in females – 0/50, 3/50 (6%), 3/50 (6%), 1/50 (2%) [not statistically significant for males or females]. [The Working Group considered that this study was adequately reported.]

3.1.2 Initiation–promotion

Groups of 20 male Swiss mice [age at start not reported; body weight, 12–15 g] were given a glyphosate-based formulation (glyphosate, 41%; polyethoxylated tallowamine, ~15%) (referred to as glyphosate in the article) that was dissolved in 50% ethanol and applied onto the shaved back skin (George *et al.*, 2010). Treatment groups were identified as follows:

- Group I – untreated control;
- Group II – glyphosate only (25 mg/kg bw), applied topically three times per week for 32 weeks;
- Group III – single topical application of dimethylbenz[*a*]anthracene (DMBA; in ethanol; 52 µg/mouse), followed 1 week later by 12-*O*-tetradecanoylphorbol-13-acetate (TPA; in acetone; 5 µg/mouse), applied topically three times per week for 32 weeks;
- Group IV – single topical application of glyphosate (25 mg/kg bw) followed 1 week later by TPA (in acetone; 5 µg/mouse), applied topically three times per week for 32 weeks;
- Group V – glyphosate (25 mg/kg bw) applied topically three times per week for 3 weeks (total of nine applications), followed 1 week later by TPA (in acetone; 5 µg/mouse), applied topically three times per week for 32 weeks;
- Group VI – single topical application of DMBA (in ethanol; 52 µg/mouse);
- Group VII –TPA (in acetone; 5 µg/mouse), applied topically three times per week for 32 weeks; and
- Group VIII –single topical application of DMBA (in ethanol; 52 µg/mouse), followed 1 week later by glyphosate (25 mg/kg bw), applied topically three times per week for 32 weeks.

All mice were killed at 32 weeks. Skin tumours were observed only in group III (positive control, DMBA + TPA, 20/20) and group VIII (DMBA + glyphosate, 8/20; $P < 0.05$ versus group VI [DMBA only, 0/20]). No microscopic examination was conducted and tumours were observed "as a minute wart like growth [that the authors called squamous cell papillomas], which progressed during the course of experiment." [The glyphosate formulation tested appeared to be a tumour promoter in this study. The design of the study was poor, with short duration of treatment, no solvent controls, small number of animals, and lack of histopathological examination. The Working Group concluded that this was an inadequate study for the evaluation of glyphosate.]

3.1.3 Review articles

Greim *et al.* (2015) have published a review article containing information on five long-term bioassay feeding studies in mice. Of these studies, one had been submitted for review to the EPA (EPA, 1985a, b, 1986, 1991a), and one to the JMPR (JMPR, 2006); these studies are discussed in Section 3.1.1. The review article reported on an additional three long-term bioassay studies in mice that had not been previously available in the open literature, but had been submitted to various organizations for registration purposes. The review article provided a brief summary of each study and referred to an online data supplement containing the original data on tumour incidence from study reports. The three additional long-term bioassay studies in mice are summarized below. [The Working Group was unable to evaluate these studies, which are not included in Table 3.1 and Section 5.3, because the information provided in the review article and its supplement was insufficient (e.g. information was lacking on statistical methods, choice of doses, body-weight gain, survival data, details of histopathological examination, and/or stability of dosed feed mixture).]

In the first study (identified as Study 12, 1997a), groups of 50 male and 50 female CD-1

mice [age at start not reported] were given diets containing glyphosate (purity, 94–96%) at a concentration of 0, 1600, 8000, or 40 000 ppm for 18 months. The increase in the incidence of bronchiolo-alveolar adenoma and carcinoma, and of lymphoma, was reported to be not statistically significant in males and females receiving glyphosate. [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

In the second study (identified as Study 13, 2001), groups of 50 male and 50 female Swiss albino mice [age at start not reported] were given diets containing glyphosate (purity, > 95%) at a concentration of 0 (control), 100, 1000, or 10 000 ppm for 18 months. The authors reported a statistically significant increase in the incidence of malignant lymphoma (not otherwise specified, NOS) in males at the highest dose: 10/50 (20%), 15/50 (30%), 16/50 (32%), 19/50 (38%; $P < 0.05$; pairwise test); and in females at the highest dose: 18/50 (36%), 20/50 (40%), 19/50 (38%), 25/50 (50%; $P < 0.05$; pairwise test). [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

In the third study (identified as Study 14, 2009a), groups of 51 male and 51 female CD-1 mice [age at start not reported] were given diets containing glyphosate (purity, 94.6–97.6%) at a concentration of 0, 500, 1500, or 5000 ppm for 18 months. Incidences for bronchiolo-alveolar adenoma and carcinoma, malignant lymphoma (NOS), and hepatocellular adenoma and carcinoma in males, and for bronchiolo-alveolar adenoma and carcinoma, malignant lymphoma (NOS) and pituitary adenoma in females, were included in the article. In males, the authors reported that there was a significant positive trend [statistical test not specified] in the incidence of bronchiolo-alveolar carcinoma (5/51, 5/51, 7/51, 11/51) and of malignant lymphoma (0/51, 1/51, 2/51, 5/51). [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

3.2 Rat

See Table 3.2

3.2.1 Drinking-water

Groups of 10 male and 10 female Sprague-Dawley rats (age, 5 weeks) were given drinking-water containing a glyphosate-based formulation at a dose of 0 (control), 1.1×10^{-8}% (5.0×10^{-5} mg/L), 0.09% (400 mg/L) or 0.5% (2.25×10^{3} mg/L), ad libitum, for 24 months (Séralini *et al.*, 2014). [The study reported is a life-long toxicology study on a glyphosate-based formulation and on genetically modified NK603 maize, which the authors stated was designed as a full study of long-term toxicity and not a study of carcinogenicity. No information was provided on the identity or concentration of other chemicals contained in this formulation.] Survival was similar in treated and control rats. [No data on body weight were provided.] In female rats, there was an almost twofold increase in the incidence of tumours of the mammary gland (mainly fibroadenoma and adenocarcinoma) in animals exposed to the glyphosate-based formulation only versus control animals: control, 5/10 (50%); lowest dose, 9/10 (90%); intermediate dose, 10/10 (100%) [$P < 0.05$; Fisher exact test]; highest dose, 9/10 (90%). [The Working Group concluded that this study conducted on a glyphosate-based formulation was inadequate for evaluation because the number of animals per group was small, the histopathological description of tumours was poor, and incidences of tumours for individual animals were not provided.]

In another study with drinking-water, Chruscielska *et al.* (2000) gave groups of 55 male and 55 female Wistar rats (age, 6–7 weeks) drinking-water containing an ammonium salt

of glyphosate as a 13.85% solution [purity of glyphosate, not reported] that was used to make aqueous solutions of 0 (control), 300, 900, and 2700 mg/L, for 24 months [details on the dosing regimen were not reported]. The authors reported that survival and body-weight gain were similar in treated and control animals. No significant increase in tumour incidence was reported in any of the treated groups. [The Working Group noted the limited information provided on dosing regimen, histopathological examination method, and tumour incidences.]

3.2.2 Dietary administration

The JMPR report included information on a 1-year feeding study in which groups of 24 male and 24 female Wistar-Alpk:APfSD rats [age at start not reported] were given diets containing glyphosate (purity, 95.6%) at a concentration of 0, 2000, 8000, or 20 000 ppm, ad libitum, for 1 year (JMPR, 2006). There was a treatment-related decrease in body-weight gain at the two highest doses (significant at 20 000 ppm for both sexes, and at 8000 ppm only in females). There was no treatment-related decrease in survival. No significant increase in tumour incidence was observed in any of the treated groups. [The Working Group noted the short duration of exposure.]

The JMPR report also included information on a 104-week feeding study in which groups of 50 male and 50 female Sprague-Dawley rats [age at start not reported] were given diets containing glyphosate (purity, 98.7–98.9%) at a concentration that was adjusted to provide doses of 0, 10, 100, 300, or 1000 mg/kg bw, ad libitum, for 104 weeks (JMPR, 2006). There was a treatment-related decrease in body-weight gain in males and females at the highest dose. There was no significant treatment-related decrease in survival or increase in tumour incidence in any of the treated groups.

Information was also included in the JMPR report on a 24-month feeding study in which groups of 52 male and 52 female Wistar-Alpk:APfSD rats [age at start not reported] were given diets containing glyphosate (purity, 97.6%) at a concentration of 0, 2000, 6000, or 20 000 ppm, ad libitum, for 24 months (JMPR, 2006). There was a treatment-related decrease in body-weight gain in males and females at the highest dose, and a corresponding significant increase in survival in males. No significant increase in tumour incidence was observed in any of the treated groups.

The EPA (1991a, b, c, d) provided information on a long-term study in which groups of 60 male and 60 female Sprague-Dawley rats (age, 8 weeks) were given diets containing glyphosate (technical grade; purity, 96.5%) at a concentration of 0 ppm, 2000 ppm, 8000 ppm, or 20 000 ppm, ad libitum, for 24 months. Ten animals per group were killed after 12 months. There was no compound-related effect on survival, and no statistically significant decreases in body-weight gain in male rats. In females at the highest dose, body-weight gain was significantly decreased, starting on day 51. In males at the lowest dose, there was a statistically significant increase in the incidence of pancreatic islet cell adenoma compared with controls: 8/57 (14%) versus 1/58 (2%), $P \leq 0.05$ (Fisher exact test). Additional analyses by the EPA (1991a) (using the Cochran–Armitage trend test and Fisher exact test, and excluding rats that died or were killed before week 55) revealed a statistically significant higher incidence of pancreatic islet cell adenoma in males at the lowest and highest doses compared with controls: lowest dose, 8/45 (18%; $P = 0.018$; pairwise test); intermediate dose, 5/49 (10%); highest dose, 7/48 (15%; $P = 0.042$; pairwise test) versus controls, 1/43 (2%). The range for historical controls for pancreatic islet cell adenoma reported in males at this laboratory was 1.8–8.5%. [The Working Group noted that there was no statistically significant positive trend in the incidence of these tumours, and no apparent progression to carcinoma.] There was also a statistically significant positive trend in the incidence of hepatocellular adenoma in

Table 3.2 Studies of carcinogenicity with glyphosate in rats

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat, Sprague-Dawley (M, F) 24 mo Séralini *et al.* (2014)	Drinking-water containing a glyphosate-based formulation at a concentration of 0 (control), 1.1×10^{-8}% (glyphosate, 5.0×10^{-5} mg/L), 0.09% (glyphosate, 400 mg/L) or 0.5% (glyphosate, 2.25×10^{3} mg/L), ad libitum, for 24 mo 10 M and 10 F/group (age, 5 wk)	*Males* No significant increase in tumour incidence observed in any of the treated groups *Females* Mammary tumours (mainly fibroadenomas and adenocarcinomas): 5/10 (50%), 9/10 (90%), 10/10 (100%)*, 9/10 (90%) Pituitary lesions (hypertrophy, hyperplasia, and adenoma): 6/10 (60%), 8/10 (80%), 7/10 (70%), 7/10 (70%)	NS *[$P < 0.05$] [NS]	Data are from an in-depth life-long toxicology study on a glyphosate-based formulation and NK603 genetically modified maize; authors stated that the study was designed as a full chronic toxicity and not a carcinogenicity study. No information provided on the identity or concentration of other chemicals contained in this formulation Histopathology poorly described and tumour incidences for individual animals not discussed in detail. Small number of animals per group [The Working Group concluded this was an inadequate study for the evaluation of glyphosate carcinogenicity]
Rat, Wistar (M, F) 24 mo Chruscielska *et al.* (2000)	Drinking-water containing ammonium salt of glyphosate (13.85% solution) [purity of glyphosate, NR] was used to make aqueous solutions of 0, 300, 900, and 2700 mg/L [Details on dosing regimen, NR] 55 M and 55 F/group (age, 6–7 wk)	No significant increase in tumour incidence observed in any of the treated groups	NS	Limited information on dosing regimen, histopathological examination methods, and tumour incidences
Rat, Wistar-Alpk:APfSD (M, F) 1 yr JMPR (2006)	Diet containing glyphosate (purity, 95.6%) at concentrations of 0, 2000, 8000, or 20 000 ppm, ad libitum, for 1 yr 24 M and 24 F/group [age, NR]	No significant increase in tumour incidence observed in any groups of treated animals	NS	Short duration of exposure
Rat, Sprague-Dawley (M, F) 104 wk JMPR (2006)	Diet containing glyphosate (purity, 98.7–98.9%) at doses of 0, 10, 100, 300, or 1000 mg/kg bw, ad libitum, for 104 wk 50 M and 50 F/group [age, NR]	No significant increase in tumour incidence observed in any groups of treated animals	NS	
Rat, Wistar-Alpk:APfSD (M, F) 24 mo JMPR (2006)	Diet containing glyphosate (purity, 97.6%) at concentrations of 0, 2000, 6000, or 20 000 ppm, ad libitum, for 2 yr 52 M and 52 F/group [age, NR]	No significant increase in tumour incidence observed in any groups of treated animals	NS	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat Sprague-Dawley (M, F) 24 mo EPA (1991a, b, c, d)	Diet containing glyphosate (technical grade; purity, 96.5%) at concentrations of 0, 2000, 8000, or 20 000 ppm, ad libitum, for 24 mo 60 M and 60 F/group (age, 8 wk) 10 rats/group killed after 12 mo	*Males* *Pancreas (islet cell):* Adenoma: 1/58 (2%), 8/57 (14%)*, 5/60 (8%), 7/59 (12%) Carcinoma: 1/58 (2%), 0/57, 0/60, 0/59 Adenoma or carcinoma (combined): 2/58 (3%), 8/57 (14%), 5/60 (8%), 7/59 (12%) *Liver:* Hepatocellular adenoma: 2/60 (3%), 2/60 (3%), 3/60 (6%), 7/60 (12%) Hepatocellular carcinoma: 3/60 (5%), 2/60 (3%), 1/60 (2%), 2/60 (3%) *Females* *Pancreas (islet cell):* Adenoma: 5/60 (8%), 1/60 (2%), 4/60 (7%), 0/59 Carcinoma: 0/60, 0/60, 0/60, 0/59 Adenoma or carcinoma (combined): 5/60 (8%), 1/60 (2%), 4/60 (7%), 0/59 *Thyroid:* C-cell adenoma: 2/60 (3%), 2/60 (3%), 6/60 (10%), 6/60 (10%) C-cell carcinoma: 0/60, 0/60, 1/60, 0/60	Adenoma, * $P \leq 0.05$ (Fisher exact test with Bonferroni inequality); see comments Adenoma, P for trend = 0.016; see comments NS Adenoma, P for trend = 0.031; see comments	Historical control range for pancreatic islet cell adenoma reported in males at this laboratory, 1.8–8.5% EPA (1991a) performed additional analyses using the Cochran–Armitage trend test and Fisher exact test, and excluding animals that died or were killed before wk 54–55: *Males* *Pancreas (islet cell):* Adenoma: 1/43 (2%), 8/45 (18%; $P = 0.018$), 5/49 (10%), 7/48 (15%; $P = 0.042$) Carcinoma: 1/43 (2%), 0/45 (0%), 0/49 (0%), 0/48 (0%) Adenoma or carcinoma (combined): 2/43 (5%), 8/45 (18%), 5/49 (10%), 7/48 (15%) [There was no statistically significant positive trend in the incidence of pancreatic tumours, and no apparent progression to carcinoma] *Liver:* Hepatocellular adenoma: 2/44 (5%; P for trend = 0.016), 2/45 (4%), 3/49 (6%), 7/48 (15%) Hepatocellular carcinoma: 3/44 (7%); 2/45 (4%), 1/49 (2%), 2/48 (4%) Hepatocellular adenoma or carcinoma (combined): 5/44 (11%), 4/45 (9%), 4/49 (8%), 9/48 (19%) [There was no apparent progression to carcinoma] *Females* *Thyroid:* C-cell adenoma: 2/57 (4%; P for trend = 0.031), 2/60 (3%), 6/59 (10%), 6/55 (11%) C-cell carcinoma: 0/57, 0/60, 1/59 (2%), 0/55 C-cell adenoma or carcinoma (combined): 2/57 (4%), 2/60 (3%), 7/59 (12%), 6/55 (11%) [There was no apparent progression to carcinoma]

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat Sprague-Dawley (M, F) Lifetime (up to 26 mo) EPA (1991a, b, c, d)	Diet containing glyphosate (purity, 98.7%) at concentrations of 0 ppm, 30 ppm (3 mg/kg bw per day), 100 ppm (10 mg/kg bw per day), 300 ppm (31 mg/kg bw per day), ad libitum, up to 26 mo 50 M and 50 F/group [age, NR]	*Males* *Pancreas (islet cell):* Adenoma: 0/50 (0%), 5/49* (10%), 2/50 (4%), 2/50 (4%) Carcinoma: 0/50 (0%), 0/49 (0%), 0/50 (0%), 1/50 (2%) Adenoma or carcinoma (combined): 0/50 (0%), 5/49 (10%), 2/50 (4%), 3/50 (6%) *Females* *Pancreas (islet cell):* Adenoma: 2/50 (4%), 1/50 (2%), 1/50 (2%), 0/50 (0%) Carcinoma: 0/50 (0%), 1/50 (2%), 1/50 (2%), 1/50 (2%) Adenoma or carcinoma (combined): 2/50 (10%), 2/50 (2%), 2/50 (74%), 1/50 (2%)	Adenoma, *[$P < 0.05$; Fisher exact test] NS	[There was no statistically significant positive trend in the incidence of pancreatic tumours, and no apparent progression to carcinoma]

bw, body weight; d, day; F, female; M, male; mo, month; NR, not reported; NS, not significant; wk, week; yr, year

males (P = 0.016) and of thyroid follicular cell adenoma in females (P = 0.031). [The Working Group noted that there was no apparent progression to carcinoma for either tumour type.]

The EPA (1991a, b, c, d) provided information on another long-term study in which groups of 50 male and 50 female Sprague-Dawley rats [age at start not reported] were given diets containing glyphosate (purity, 98.7%) at a concentration of 0, 30 (3 mg/kg bw per day), 100 (10 mg/kg bw per day), or 300 ppm (31 mg/kg bw per day), ad libitum, for life (up to 26 months). No information was provided on body weight or survival of the study animals. An increase in the incidence of pancreatic islet cell adenoma was reported in males at the lowest dose: controls, 0/50 (0%); lowest dose, 5/49 (10%) [P < 0.05; Fisher exact test]; intermediate dose, 2/50 (4%); highest dose, 2/50 (4%). [The Working Group noted that there was no statistically significant positive dose-related trend in the incidence of these tumours, and no apparent progression to carcinoma.]

3.2.3 Review articles

Greim *et al.* (2015) have published a review article containing information on nine long-term bioassay feeding studies in rats. Of these studies, two had been submitted for review to the EPA (1991a, b, c, d), two to the JMPR (JMPR, 2006), and one had been published in the openly available scientific literature (Chruscielska *et al.*, 2000); these studies are discussed earlier in Section 3.2. The review article reported on an additional four long-term bioassay studies in rats that had not been previously published, but had been submitted to various organizations for registration purposes. The review article provided a brief summary of each study and referred to an online data supplement containing the original data on tumour incidence from study reports. The four additional long-term bioassay studies in rats are summarized below. [The Working Group did not evaluate these studies, which are not included in Table 3.2 and Section 5.3, because the information provided in the review article and its supplement was insufficient (e.g. information lacking on statistical methods, choice of doses, body-weight gain, survival data, details on histopathological examination and/or stability of dosed feed mixture).]

In one study (identified as Study 4, 1996), groups of 50 male and 50 female Wistar rats [age at start not reported] were given diets containing glyphosate (purity, 96%) at a concentration of 0, 100, 1000, or 10 000 ppm, ad libitum, for 24 months. It was reported that hepatocellular adenomas and hepatocellular carcinomas were found at non-statistically significant incidences in both males and females. There was no significant increase in tumour incidence in the treated groups. [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

In one study in Sprague-Dawley rats (identified as Study 5, 1997), groups of 50 male and 50 female rats [age at start not reported] were given diets containing glyphosate technical acid [purity not reported] at a concentration of 0, 3000, 15 000, or 25 000 ppm, ad libitum, for 24 months. There was no significant increase in tumour incidence in the treated groups. [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

In a second study in Sprague Dawley rats (identified as Study 6, 1997b), groups of 50 males and 50 females [age at start not reported] were given diets containing glyphosate (purity, 94.6–97.6%) at a concentration of 0, 3000, 10 000, or 30 000 ppm, ad libitum, for 24 months. Non-significant increases in tumour incidences compared with controls were noted for skin keratoacanthoma in males at the highest dose, and for fibroadenoma of the mammary gland in females at the lowest and intermediate doses. [The Working Group was unable to evaluate this

study because of the limited experimental data provided in the review article and supplemental information.]

In another study in male and female Wistar rats (identified as Study 8, 2009b), groups of 51 male and 51 female rats [age at start not reported] were fed diets containing glyphosate (purity, 95.7%) at a concentration of 0, 1500, 5000, or 15 000 ppm, ad libitum, for 24 months. The highest dose was progressively increased to reach 24 000 ppm by week 40. A non-significant increase in tumour incidence was noted for adenocarcinoma of the mammary gland in females at the highest dose (6/51) compared with controls (2/51). [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information. The Working Group noted that tumours of the mammary gland had been observed in other studies in rats reviewed for the present *Monograph*.]

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

4.1.1 Introduction

The herbicidal activity of glyphosate is attributed to interference with the production of essential aromatic amino acids (EPA, 1993b). In plants, glyphosate competitively inhibits the activity of enolpyruvylshikimate phosphate synthase, an enzyme that is not present in mammalian cells. Glyphosate is degraded by soil microbes to aminomethylphosphonic acid (AMPA) (see Fig. 4.1), a metabolite that can accumulate in the environment. In mammals, glyphosate is not metabolized efficiently, and is mainly excreted unchanged into the urine; however, it has been suggested that glyphosate can undergo gut microbial metabolism in humans (Motojyuku *et al.*, 2008) and rodents (Brewster *et al.*, 1991).

4.1.2 Absorption

(a) Humans

Data on the absorption of glyphosate via intake of food and water in humans were not available to the Working Group. Inhalation of glyphosate is considered to be a minor route of exposure in humans, because glyphosate is usually formulated as an isopropylamine salt with a very low vapour pressure (Tomlin, 2000).

In the Farm Family Exposure Study, 60% of farmers had detectable levels of glyphosate in 24-hour composite urine samples taken on the day they had applied a glyphosate-based formulation (Acquavella *et al.*, 2004). Farmers who did not use rubber gloves had higher urinary concentrations of glyphosate than those who did use gloves [indicating that dermal absorption is a relevant route of exposure]. In a separate study, detectable levels of glyphosate were found in urine samples from farm families and non-farm families (Curwin *et al.*, 2007).

In accidental and deliberate intoxication cases involving ingestion of glyphosate-based formulations, glyphosate was readily detectable in the blood (Zouaoui *et al.*, 2013). After deliberate or accidental ingestion, one glyphosate-based formulation was found to be more lethal to humans than another (Sørensen & Gregersen, 1999). [Greater lethality was attributed to the presence of trimethylsulfonium counterion, which might facilitate greater absorption after oral exposure.]

Small amounts of glyphosate can be absorbed after dermal exposures in humans in vitro. For example, when an aqueous solution of 1% glyphosate was applied in an in-vitro human skin model, only 1.4% of the applied dose was absorbed through the skin. Glyphosate is typically formulated as an isopropylamine salt, and is dissolved in a water-based vehicle, while the

stratum corneum is a lipid-rich tissue (Wester *et al.*, 1991). In-vitro studies using human skin showed that percutaneous absorption of a glyphosate-based formulation was no more than 2% of the administered dose over a concentration range of 0.5–154 μg/cm² and a topical volume range of 0.014–0.14 mL/cm². In addition, very little glyphosate (≤ 0.05% of the administered dose) was sequestered in the stratum corneum after dermal application (Wester *et al.*, 1991).

In the human Caco-2 cell line, an in-vitro model of intestinal enterocytes, glyphosate (> 10 mg/mL) was shown to significantly disrupt barrier properties, leading to an increase in paracellular permeability (transport of substances that pass through the intercellular space between the cells) (Vasiluk *et al.*, 2005).

(b) Experimental systems

Three studies have been conducted to investigate the absorption of a single oral dose of glyphosate in rats (Brewster *et al.*, 1991; Chan & Mahler, 1992; EPA, 1993b).

In male Sprague-Dawley rats given [^{14}C]-labelled glyphosate (10 mg/kg bw), the majority of the radiolabel was associated with the gastrointestinal contents and small intestinal tissue 2 hours after administration (Brewster *et al.*, 1991). Approximately 35–40% of the administered dose was found to be absorbed from the gastrointestinal tract. Urinary and faecal routes of elimination were equally important. [The Working Group concluded that glyphosate is incompletely absorbed from the gastrointestinal tract after oral exposure in rats.]

In a study by the United States National Toxicology Programme (NTP) in Fisher 344 rats, 30% of the administered oral dose (5.6 mg/kg bw) was absorbed, as determined by urinary excretion data (Chan & Mahler, 1992). This finding was in accordance with the previously described study of oral exposure in rats (Brewster *et al.*, 1991).

In a study reviewed by the EPA, Sprague-Dawley rats were given an oral dose of glyphosate (10 mg/kg bw); 30% and 36% of the administered dose was absorbed in males and females, respectively (EPA, 1993b). At a dose that was ~10-fold higher (1000 mg/kg bw), oral absorption of glyphosate by the rats was slightly reduced.

In a 14-day feeding study in Wistar rats given glyphosate at dietary concentrations of up to 100 ppm, only ~15% of the administered dose was found to be absorbed (JMPR, 2006). In New Zealand White rabbits or lactating goats given glyphosate as single oral doses (6–9 mg/kg bw), a large percentage of the administered dose was recovered in the faeces [suggesting very poor gastrointestinal absorption of glyphosate in these animal models] (JMPR, 2006).

In monkeys given glyphosate by dermal application, percutaneous absorption was estimated to be between 1% and 2% of the administered dose (Wester *et al.*, 1991). Most of the administered dose was removed by surface washes of the exposed skin.

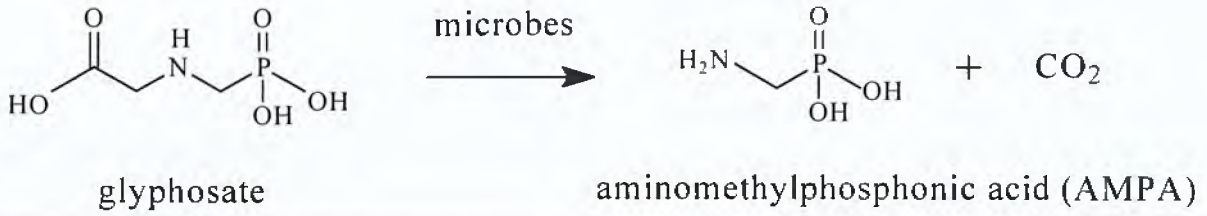
4.1.3 Distribution

(a) Humans

No data in humans on the distribution of glyphosate in systemic tissues other than blood were available to the Working Group. In cases of accidental or deliberate intoxication involving ingestion of glyphosate-based formulations, glyphosate was measured in blood. Mean blood concentrations of glyphosate were 61 mg/L and 4146 mg/L in mild-to-moderate cases of intoxication and in fatal cases, respectively (Zouaoui *et al.*, 2013).

One report, using optical spectroscopy and molecular modelling, indicated that glyphosate could bind to human serum albumin, mainly by hydrogen bonding; however, the fraction of glyphosate that might bind to serum proteins in blood was not actually measured (Yue *et al.*, 2008).

Fig. 4.1 Microbial metabolism of glyphosate to AMPA



Glyphosate is degraded to AMPA by microbial metabolism
Compiled by the Working Group

(b) Experimental systems

In Sprague-Dawley rats given a single oral dose of glyphosate (100 mg/kg bw), glyphosate concentrations in plasma reached peak levels, then declined slowly from day 1 to day 5 (Bernal *et al.*, 2010). The plasma data appeared to fit a one-compartment model with an elimination rate constant of k_{el} = 0.021 hour^{-1}. [The Working Group estimated the elimination half-life of glyphosate to be 33 hours.] Tissue levels of glyphosate were not determined in this study. In a study by Brewster *et al.* (1991), the tissue levels of glyphosate at 2, 6.3, 28, 96, and 168 hours in Sprague-Dawley rats given a single oral dose (10 mg/kg bw) declined rapidly. Tissues with the greatest amounts of detectable radiolabel (> 1% of the administered dose) were the small intestine, colon, kidney, and bone. Peak levels were reached in small intestine tissue and blood by 2 hours, while peak levels in other tissues occurred at 6.3 hours after dosing. After 7 days, the total body burden of [^{14}C]-labelled residues was ~1% of the administered dose, and was primarily associated with the bone (~1 ppm). In every tissue examined after administration of [^{14}C]-labelled glyphosate, essentially 100% of the radiolabel that was present in the tissue was unmetabolized parent glyphosate. Thus, essentially 100% of the body burden was parent compound, with no significant persistence of glyphosate after 7 days (Brewster *et al.*, 1991). In a 14-day feeding study in Wistar rats given diets containing glyphosate at 100 ppm, glyphosate reached steady-state levels in the blood by day 6 (JMPR, 2006). The tissue concentrations of glyphosate had the following rank order: kidneys > spleen > fat > liver. Tissue levels declined rapidly after cessation of exposure to glyphosate. A second study in rats given glyphosate (10 mg/kg bw per day, 14 days) followed by a single oral dose of [^{14}C]-glyphosate (at 10 mg/kg bw) showed that repeated dosing did not alter the tissue distribution of glyphosate (JMPR, 2006).

In rhesus monkeys, tissues harvested 7 days after dermal exposures to [^{14}C]-labelled glyphosate did not contain radiolabel at detectable levels (Wester *et al.*, 1991).

4.1.4 Metabolism and modulation of metabolic enzymes

(a) Metabolism

Glyphosate is degraded in the environment by soil microbes, primarily to AMPA and carbon dioxide (Fig. 4.1; Jacob *et al.*, 1988). A minor pathway for the degradation of glyphosate in bacteria (*Pseudomonas sp.* strain LBr) is via conversion to glycine (Jacob *et al.*, 1988). In a case of deliberate poisoning with a glyphosate-based formulation, small amounts of AMPA (15.1 µg/mL) were detectable in the blood (Motojyuku *et al.*, 2008) [suggesting that this pathway might also operate in humans]. In rats given a single high oral dose of glyphosate (100 mg/kg bw), small amounts of AMPA were detected in the plasma (Bernal *et al.*, 2010). In

male Sprague-Dawley rats given an oral dose of glyphosate (10 mg/kg bw), a very small amount of AMPA (< 0.04% of the administered dose) was detected in the colon 2 hours after dosing; this was attributed to intestinal microbial metabolism (Brewster *et al.*, 1991).

(b) Modulation of metabolic enzymes

(i) Humans

In human hepatic cell lines, treatment with one of four glyphosate-based formulations produced by the same company was shown to enhance CYP3A4 and CYP1A2 levels, while glutathione transferase levels were reduced (Gasnier *et al.*, 2010). [The Working Group noted that it was not clear whether the effects were caused by glyphosate alone or by the adjuvants contained in the formulation.]

(ii) Experimental systems

Exposure of Wistar rats to a glyphosate-based formulation significantly altered some hepatic xenobiotic enzyme activities (Larsen *et al.*, 2014). Liver microsomes obtained from male and female rats treated with the formulation exhibited ~50% reductions in cytochrome P450 (CYP450) content compared with control (untreated) rats. However, opposing effects were observed when assessing 7-ethoxycoumarin *O*-deethylase activity (7-ECOD, a non-specific CYP450 substrate). Female rats treated with the glyphosate-based formulation exhibited a 57% increase in hepatic microsomal 7-ECOD activity compared with controls, while male rats treated with the formulation exhibited a 58% decrease in this activity (Larsen *et al.*, 2014). [The Working Group noted that it was not clear whether the effects were caused by glyphosate alone or by adjuvants contained in the formulation.]

4.1.5 Excretion

(a) Humans

Excretion of glyphosate in humans was documented in several biomonitoring studies. For example, as part of the Farm Family Exposure Study, urinary concentrations of glyphosate were evaluated immediately before, during, and after glyphosate application in 48 farmers and their spouses and children (Acquavella *et al.*, 2004). Dermal contact with glyphosate during mixing, loading, and application was considered to be the main route of exposure in the study. On the day the herbicide was applied, 60% of the farmers had detectable levels of glyphosate in 24-hour composite urine samples, as did 4% of their spouses and 12% of children. For farmers, the geometric mean concentration was 3 µg/L, the maximum value was 233 µg/L, and the highest estimated systemic dose was 0.004 mg/kg bw (Acquavella *et al.*, 2004). In a separate study, detectable levels of glyphosate were excreted in the urine of members of farm families and of non-farm families, with geometric means ranging from 1.2 to 2.7 µg/L (Curwin *et al.*, 2007).

In a study of a rural population living near areas sprayed for drug eradication in Colombia (see Section 1.4.1, Table 1.5), mean urinary glyphosate concentrations were 7.6 µg/L (range, undetectable to 130 µg/L) (Varona *et al.*, 2009). AMPA was detected in 4% of urine samples (arithmetic mean, 1.6 µg/L; range, undetectable to 56 µg/L).

(b) Experimental systems

In an NTP study in Fisher 344 rats given a single oral dose of [^{14}C]-labelled glyphosate (5.6 or 56 mg/kg bw), it was shown that > 90% of the radiolabel was eliminated in the urine and faeces within 72 hours (Chan & Mahler, 1992). In Sprague-Dawley rats given [^{14}C]-labelled glyphosate at an oral dose of 10 or 1000 mg/kg bw, ~60–70% of the administered dose was excreted in the faeces, and the remainder in the urine (EPA,

1993b). By either route, most (98%) of the administered dose was excreted as unchanged parent compound. AMPA was the only metabolite found in the urine (0.2–0.3% of the administered dose) and faeces (0.2–0.4% of the administered dose). [The large amount of glyphosate excreted in the faeces is consistent with its poor oral absorption.] Less than 0.3% of the administered dose was expired as carbon dioxide.

In rhesus monkeys given glyphosate as an intravenous dose (9 or 93 μg), > 95% of the administered dose was excreted in the urine (Wester *et al.*, 1991). Nearly all the administered dose was eliminated within 24 hours. In contrast, in rhesus monkeys given glyphosate by dermal application (5400 μg/20 cm^2), only 2.2% of the administered dose was excreted in the urine within 7 days (Wester *et al.*, 1991).

Overall, systemically absorbed glyphosate is not metabolized efficiently, and is mainly excreted unchanged into the urine.

4.2 Mechanisms of carcinogenesis

4.2.1 Genetic and related effects

Glyphosate has been studied for genotoxic potential in a wide variety of assays. Studies carried out in exposed humans, in human cells in vitro, in other mammals in vivo and in vitro, and in non-mammalian systems in vivo and in vitro, respectively, are summarized in Table 4.1, Table 4.2, Table 4.3, Table 4.4, and Table 4.5. [A review article by Kier & Kirkland (2013) summarized the results of published articles and unpublished reports of studies pertaining to the genotoxicity of glyphosate and glyphosate formulations. A supplement to this report contained information on 66 unpublished regulatory studies. The conclusions and data tables for each individual study were included in the supplement; however, the primary study reports from which these data were extracted were not available to the Working Group. The information provided in the supplement was insufficient regarding topics such as details of statistical methods, choice of the highest dose tested, and verification of the target tissue exposure. The Working Group determined that the information in the supplement to Kier & Kirkland (2013) did not meet the criteria for data inclusion as laid out in the Preamble to the *IARC Monographs*, being neither "reports that have been published or accepted for publication in the openly available scientific literature" nor "data from governmental reports that are publicly available" (IARC, 2006). The review article and supplement were not considered further in the evaluation.]

(a) Humans

(i) Studies in exposed humans

See Table 4.1

In exposed individuals (n = 24) living in northern Ecuador in areas sprayed with a glyphosate-based formulation, a statistically significant increase in DNA damage (DNA strand breaks) was observed in blood cells collected 2 weeks to 2 months after spraying (Paz-y-Miño *et al.*, 2007). The same authors studied blood cells from individuals (n = 92) in 10 communities in Ecuador's northern border, who were sampled 2 years after the last aerial spraying with a herbicide mix containing glyphosate, and showed that their karyotypes were normal compared with those of a control group (Paz-y-Miño *et al.*, 2011).

Bolognesi *et al.* (2009) studied community residents (137 women of reproductive age and their 137 spouses) from five regions in Colombia. In three regions with exposures to glyphosate-based formulations from aerial spraying, blood samples were taken from the same individuals at three time-points (before spraying (baseline), 5 days after spraying and 4 months after spraying) to determine the frequency of micronucleus formation in lymphocytes. The baseline frequency of binucleated cells with micronuclei was significantly higher in subjects

from the three regions where there had been aerial spraying with glyphosate-formulations and in a fourth region with pesticide exposure (but not through aerial spraying), compared with a reference region (without use of pesticide). The frequency of micronucleus formation in peripheral blood lymphocytes was significantly increased, compared with baseline levels in the same individuals, after aerial spraying with glyphosate-based formulations in each of the three regions (see Table 4.1; Bolognesi *et al.*, 2009). Immediately after spraying, subjects who reported direct contact with the glyphosate-based spray showed a higher frequency of binucleated cells with micronuclei. However, the increase in frequency of micronucleus formation observed immediately after spraying was not consistent with the rates of application used in the regions, and there was no association between self-reported direct contact with pesticide sprays and frequency of binucleated cells with micronuclei. In subjects from one but not other regions, the frequency of binucleated cells with micronuclei was significantly decreased 4 months after spraying, compared with immediately after spraying.

(ii) Human cells in vitro

See Table 4.2

Glyphosate induced DNA strand breaks (as measured by the comet assay) in liver Hep-2 cells (Mañas *et al.*, 2009a), lymphocytes (Mladinic *et al.*, 2009b; Alvarez-Moya *et al.*, 2014), GM38 fibroblasts, the HT1080 fibrosarcoma cell line (Monroy *et al.*, 2005), and the TR146 buccal carcinoma line (Koller *et al.*, 2012). DNA strand breaks were induced by AMPA in Hep-2 cells (Mañas *et al.*, 2009b), and by a glyphosate-based formulation in the TR146 buccal carcinoma cell line (Koller *et al.*, 2012).

In human lymphocytes, AMPA (Mañas *et al.*, 2009b), but not glyphosate (Mañas *et al.*, 2009a), produced chromosomal aberrations. Glyphosate did not induce a concentration-related increase in micronucleus formation in human lymphocytes at levels estimated to correspond to occupational and residential exposure (Mladinic *et al.*, 2009a). Sister-chromatid exchange was induced by glyphosate (Bolognesi *et al.*, 1997), and by a glyphosate-based formulation (Vigfusson & Vyse, 1980; Bolognesi *et al.*, 1997) in human lymphocytes exposed in vitro.

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.3

The ability of glyphosate or a glyphosate-based formulation to induce DNA adducts was studied in mice given a single intraperitoneal dose. Glyphosate induced DNA adducts (8-hydroxy deoxyguanosine) in the liver, but not in the kidney, while a glyphosate-based formulation caused a slight increase in DNA adducts in the kidney, but not in the liver (Bolognesi *et al.*, 1997). Peluso *et al.* (1998) showed that a glyphosate-based formulation (glyphosate, 30.4%), but not glyphosate alone, caused DNA adducts (as detected by ^{32}P-DNA post-labelling) in mouse liver and kidney. Glyphosate and a glyphosate-based formulation produced DNA strand breaks in the liver and kidney after a single intraperitoneal dose (Bolognesi *et al.*, 1997).

In mice given a single dose of glyphosate by gavage, no genotoxic effect was observed by the dominant lethal test (EPA, 1980a).

After a single intraperitoneal dose, no chromosomal aberrations were observed in the bone marrow of rats treated with glyphosate (Li & Long, 1988), while chromosomal aberrations were increased in the bone marrow of mice given a glyphosate-based formulation (glyphosate isopropylamine salt, ~41%) (Prasad *et al.*, 2009). A single oral dose of a glyphosate-based formulation did not cause chromosomal aberrations in mice (Dimitrov *et al.*, 2006).

In mice treated by intraperitoneal injection, a single dose of glyphosate did not cause

micronucleus formation in the bone marrow (Rank *et al.*, 1993), although two daily doses did (Bolognesi *et al.*, 1997; Mañas *et al.*, 2009a). AMPA, the main metabolite of glyphosate, also produced micronucleus formation after two daily intraperitoneal doses (Mañas *et al.*, 2009b). Conflicting results for micronucleus induction were obtained in mice exposed intraperitoneally to a glyphosate-based formulation. A single dose of the formulation at up to 200 mg/kg bw did not induce micronucleus formation in the bone marrow in one study (Rank *et al.* 1993), while it did increase micronucleus formation at 25 mg/kg bw in another study (Prasad *et al.*, 2009). After two daily intraperitoneal doses, a glyphosate-based formulation did not induce micronucleus formation at up to 200 mg/kg bw according to Grisolia (2002), while Bolognesi *et al.* (1997) showed that the formulation did induce micronucleus formation at 450 mg/kg bw. In mice given a single oral dose of a glyphosate-based formulation at 1080 mg/kg bw, no induction of micronuclei was observed (Dimitrov *et al.*, 2006).

(ii) Non-human mammalian cells in vitro

See Table 4.4

Glyphosate did not induce unscheduled DNA synthesis in rat primary hepatocytes, or *Hprt* mutation (with or without metabolic activation) in Chinese hamster ovary cells (Li & Long, 1988).

In bovine lymphocytes, chromosomal aberrations were induced by glyphosate in one study (Lioi *et al.*, 1998), but not by a glyphosate formulation in another study (Siviková & Dianovský, 2006). Roustan *et al.* (2014) demonstrated, in the CHO-K1 ovary cell line, that glyphosate induced micronucleus formation only in the presence of metabolic activation, while AMPA induced micronucleus formation both with and without metabolic activation. Sister-chromatid exchange was observed in bovine lymphocytes exposed to glyphosate (Lioi *et al.*, 1998) or a glyphosate formulation (in the absence but not the presence of metabolic activation) (Siviková & Dianovský, 2006).

(iii) Non-mammalian systems in vivo

See Table 4.5

Fish and other species

In fish, glyphosate produced DNA strand breaks in the comet assay in sábalo (Moreno *et al.*, 2014), European eel (Guilherme *et al.*, 2012b), zebrafish (Lopes *et al.*, 2014), and Nile tilapia (Alvarez-Moya *et al.*, 2014). AMPA also induced DNA strand breaks in the comet assay in European eel (Guilherme *et al.*, 2014b). A glyphosate-based formulation produced DNA strand breaks in numerous fish species, such as European eel (Guilherme *et al.*, 2010, 2012b, 2014a; Marques *et al.*, 2014, 2015), sábalo (Cavalcante *et al.*, 2008; Moreno *et al.*, 2014), guppy (De Souza Filho *et al.*, 2013), bloch (Nwani *et al.*, 2013), neotropical fish *Corydoras paleatus* (de Castilhos Ghisi & Cestari, 2013), carp (Gholami-Seyedkolaei *et al.*, 2013), and goldfish (Cavaş & Könen, 2007).

AMPA, the main metabolite of glyphosate, induced erythrocytic nuclear abnormalities (kidney-shaped and lobed nuclei, binucleate or segmented nuclei and micronuclei) in European eel (Guilherme *et al.*, 2014b). Micronucleus formation was induced by different glyphosate-based formulations in various fish (Grisolia, 2002; Cavaş & Könen, 2007; De Souza Filho *et al.*, 2013; Vera-Candioti *et al.*, 2013).

Glyphosate-based formulations induced DNA strand breaks in other species, including caiman (Poletta *et al.*, 2009), frog (Meza-Joya *et al.*, 2013), tadpoles (Clements *et al.*, 1997), and snail (Mohamed, 2011), but not in oyster (Akcha *et al.*, 2012), clam (dos Santos & Martinez, 2014), and mussel glochidia (Conners & Black, 2004). In earthworms, one glyphosate-based formulation induced DNA strand breaks while two others did not (Pioła *et al.*, 2013; Muangphra *et al.*, 2014), highlighting the potential importance of components other than the active ingredient in the formulation.

Table 4.1 Genetic and related effects of glyphosate in exposed humans

Tissue	Cell type (if specified)	End-point	Test	Description of exposure and controls	Response[a]/ significance	Comments	Reference
Blood	NR	DNA damage	DNA strand breaks, comet assay	24 exposed individuals in northern Ecuador; areas sprayed with glyphosate-based formulation (sampling 2 weeks to 2 months after spraying); control group was 21 non-exposed individuals	+ $P < 0.001$		Paz-y-Miño *et al.* (2007)
Blood	NR	Chromosomal damage	Chromosomal aberrations	92 individuals in 10 communities, northern border of Ecuador; sampling 2 years after last aerial spraying with herbicide mix containing glyphosate); control group was 90 healthy individuals from several provinces without background of smoking or exposure to genotoxic substances (hydrocarbons, X-rays, or pesticides)	–	182 karyotypes were considered normal [Smoking status, NR]	Paz-y-Miño *et al.* (2011)
Blood	Lymphocytes	Chromosomal damage	Micronucleus formation	55 community residents, Nariño, Colombia; area with aerial glyphosate-based formulation spraying for coca and poppy eradication (glyphosate was tank-mixed with an adjuvant)	+ [$P < 0.001$]	P values for after spraying vs before spraying in the same individuals	Bolognesi *et al.* (2009)
Blood	Lymphocytes	Chromosomal damage	Micronucleus formation	53 community residents, Putumayo, Colombia; area with aerial glyphosate-based formulation spraying for coca and poppy eradication (glyphosate was tank-mixed with an adjuvant)	+ [$P = 0.01$]	P values for after spraying vs before spraying in the same individuals	Bolognesi *et al.* (2009)
Blood	Lymphocytes	Chromosomal damage	Micronucleus formation	27 community residents, Valle del Cauca, Colombia; area where glyphosate-based formulation was applied through aerial spraying for sugar-cane maturation (glyphosate was applied without adjuvant)	+ [$P < 0.001$]	P values for after spraying vs before spraying in the same individuals	Bolognesi *et al.* (2009)

[a] +, positive; –, negative
NR, not reported; vs, versus

Table 4.2 Genetic and related effects of glyphosate, AMPA, and glyphosate-based formulations in human cells in vitro

Tissue, cell line	End-point	Test	Results[a]		Dose (LED or HID)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Glyphosate							
Liver Hep-2	DNA damage	DNA strand breaks, comet assay	+	NT	3 mM [507.2 µg/mL]	$P < 0.01$; dose–response relationship ($r \geq 0.90$; $P < 0.05$)	Mañas et al. (2009a)
Lymphocytes	DNA damage	DNA strand breaks, standard and hOGG1 modified comet assay	+	+	3.5 µg/mL	With the hOGG1 modified comet assay, + S9, the increase was significant ($P < 0.01$) only at the highest dose tested (580 µg/mL)	Mladinic *et al.* (2009b)
Lymphocytes	DNA damage	DNA strand breaks, comet assay	+	NT	0.0007 mM [0.12 µg/mL]	$P \leq 0.01$	Alvarez-Moya *et al.* (2014)
Fibroblast GM 38	DNA damage	DNA strand breaks, comet assay	+	NT	4 mM [676 µg/mL]	$P < 0.001$	Monroy *et al.* (2005)
Fibroblast GM 5757	DNA damage	DNA strand breaks, comet assay	(+)	NT	75 mM [12 680 µg/mL]	Glyphosate (ineffective alone, data NR) increased strand breaks induced by H_2O_2 (40 or 50 µM) ($P < 0.004$ vs H_2O_2 alone)	Lucken *et al.* (2004)
Fibrosarcoma HT1080	DNA damage	DNA strand breaks, comet assay	+	NT	4.75 mM [803 µg/mL]	$P < 0.001$	Monroy *et al.* (2005)
Buccal carcinoma TR146	DNA damage	DNA strand breaks, SCGE assay	+	NT	20 µg/mL	Dose-dependent increase ($P \leq 0.05$)	Koller *et al.* (2012)
Lymphocytes	Chromosomal damage	Chromosomal aberrations	–	NT	6 mM [1015 µg/mL]		Mañas *et al.* (2009a)
Lymphocytes	Chromosomal damage	Micronucleus formation	–	(+)	580 µg/mL	$P < 0.01$ at the highest exposure + S9 No concentration-related increase in micronuclei containing the centromere signal (C+)	Mladinic *et al.* (2009a)

Table 4.2 (continued)

Tissue, cell line	End-point	Test	Results[a]		Dose (LED or HID)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	1000 µg/mL	$P < 0.05$	Bolognesi *et al.* (1997)
AMPA							
Liver Hep-2	DNA damage	DNA strand breaks, comet assay	+	NT	4.5 mM [500 µg/mL]	$P < 0.05$ at 4.5 mM; $P < 0.01$ at up to 7.5 mM Dose–response relationship ($r \geq 0.90$; $P < 0.05$)	Mañas *et al.* (2009b)
Lymphocytes	Chromosomal damage	Chromosomal aberrations	+	NT	1.8 mM [200 µg/mL]	$P < 0.05$	Mañas *et al.* (2009b)
Glyphosate-based formulations							
Liver HepG2	DNA damage	DNA strand breaks, comet assay	(+)	NT	5 ppm	Glyphosate, 400 g/L Dose-dependent increase; greatest increase at 10 ppm Statistical analysis, NR	Gasnier *et al.* (2009)
Buccal carcinoma TR146	DNA damage	DNA strand breaks, SCGE assay	+	NT	20 µg/mL	Glyphosate acid, 450g/L Dose-dependent increase ($P \leq 0.05$)	Koller *et al.* (2012)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	250 µg/mL	$P < 0.001$ No growth at 25 mg/mL	Vigfusson & Vyse (1980)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	100 µg/mL	Glyphosate, 30.4% $P < 0.05$	Bolognesi *et al.* (1997)

[a] +, positive; -, negative; (+) or (-) positive/negative in a study with limited quality

AMPA, aminomethyl phosphonic acid; HID, highest ineffective dose; hOGG1, human 8-hydroxyguanosine DNA-glycosylase; LED, lowest effective dose; NR, not reported; NT, not tested; S9, 9000 × *g* supernatant; SCGE, single cell gel electrophoresis; vs, versus

Micronucleus formation was induced by a glyphosate-based formulation (glyphosate, 36%) in earthworms (Muangphra *et al.*, 2014), and by a different glyphosate-based formulation in caiman (Poletta *et al.*, 2009, 2011), and frog (Yadav *et al.*, 2013).

Insects

In standard *Drosophila melanogaster*, glyphosate induced mutation in the test for somatic mutation and recombination, but not in a cross of flies characterized by an increased capacity for CYP450-dependent bioactivation (Kaya *et al.*, 2000). A glyphosate-based formulation also caused sex-linked recessive lethal mutations in *Drosophila* (Kale *et al.*, 1995).

Plants

In plants, glyphosate produced DNA damage in *Tradescantia* in the comet assay (Alvarez-Moya *et al.*, 2011). Chromosomal aberration was induced after exposure to glyphosate in fenugreek (Siddiqui *et al.*, 2012), and in onion in one study (Frescura *et al.*, 2013), but not in another (Rank *et al.*, 1993). A glyphosate-based formulation also induced chromosomal aberration in barley roots (Truta *et al.*, 2011) and onion (Rank *et al.*, 1993), but not in *Crepis capillaris* (hawksbeard) (Dimitrov *et al.*, 2006). Micronucleus formation was not induced by glyphosate in *Vicia faba* bean (De Marco *et al.*, 1992) or by a glyphosate-based formulation in *Crepis capillaris* (Dimitrov *et al.*, 2006).

(iv) Non-mammalian systems in vitro

See Table 4.6

Glyphosate induced DNA strand breaks in erythrocytes of tilapia fish, as demonstrated by comet assay (Alvarez-Moya *et al.*, 2014).

Glyphosate did not induce mutation in *Bacillus subtillis*, *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100, or in *Escherichia coli* WP2, with or without metabolic activation (Li & Long, 1988). However, Rank *et al.* (1993) demonstrated that a glyphosate-based formulation was mutagenic in *S. typhimurium* TA98 in the absence of metabolic activation, and in *S. typhimurium* TA100 in the presence of metabolic activation.

4.2.2 Receptor-mediated mechanisms

(a) Sex-hormone pathway disruption

(i) Humans

Studies in exposed humans

No data were available to the Working Group.

Human cells in vitro

In hormone-dependent T47D breast cancer cells, the proliferative effects of glyphosate (10^{-6} to 1 μM) (see Section 4.2.4) and those of 17β-estradiol (the positive control) were mitigated by the estrogen receptor antagonist, ICI 182780; the proliferative effect of glyphosate was completely abrogated by the antagonist at a concentration of 10 nM (Thongprakaisang *et al.*, 2013). Glyphosate also induced activation of the estrogen response element (ERE) in T47D breast cancer cells that were stably transfected with a triplet ERE-promoter-luciferase reporter gene construct. Incubation with ICI 182780 at 10 nM eliminated the response. When the transfected cells were incubated with both 17β-estradiol and glyphosate, the effect of 17β-estradiol was reduced and glyphosate behaved as an estrogen antagonist. After 6 hours of incubation, glyphosate increased levels of estrogen receptors ERα and ERβ in a dose-dependent manner in T47D cells; after 24 hours, only ERβ levels were increased and only at the highest dose of glyphosate. [These findings suggested that the proliferative effects of glyphosate on T47D cells are mediated by ER.]

In human hepatocarcinoma HepG2 cells, four glyphosate-based formulations produced by the same company had a marked effect on the activity and transcription of aromatase, while glyphosate alone differed from controls, but not significantly so (Gasnier *et al.*, 2009).

Table 4.3 Genetic and related effects of glyphosate, AMPA, and glyphosate-based formulations in non-human mammals in vivo

Species, strain (sex)	Tissue	End-point	Test	Results	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Glyphosate								
Mouse, Swiss CD1 (M)	Liver	DNA damage	DNA adducts, 8-OHdG by LC/UV	+	300 mg/kg bw	i.p.; 1×; sampled after 8 and 24 h	Single dose tested only $P < 0.05$ after 24 h	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M)	Kidney	DNA damage	DNA adducts, 8-OHdG by LC/UV	–	300 mg/kg bw	i.p.; 1×; sampled after 8 and 24 h	Single dose tested only	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M, F)	Kidney	DNA damage	DNA adducts, ^{32}P-DNA post labelling	–	270 mg/kg bw	i.p.; 1 ×; sampled after 24 h	Glyphosate isopropylammonium salt	Peluso *et al.* (1998)
Mouse, Swiss CD1 (M, F)	Liver	DNA damage	DNA adducts, ^{32}P-DNA post labelling	–	270 mg/kg bw	i.p.; 1 ×; sampled after 24 h	Glyphosate isopropylammonium salt	Peluso *et al.* (1998)
Mouse, Swiss CD1 (M)	Liver	DNA damage	DNA strand breaks, alkaline elution assay	+	300 mg/kg bw	i.p.; 1 ×; sampled after 4 and 24 h	Single dose tested only $P < 0.05$ after 4 h	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M)	Kidney	DNA damage	DNA strand breaks, alkaline elution assay	+	300 mg/kg bw	i.p.; 1 ×; sampled after 4 and 24 h	Single dose tested only $P < 0.05$ after 4 h	Bolognesi *et al.* (1997)
Mouse, CD-1 (M)	Uterus after mating	Mutation	Dominant lethal test	–	2000 mg/kg bw	Oral gavage; 1 ×	Proportion of early resorptions evaluated after mating of non-treated females with glyphosate-treated male mice	EPA (1980)
Rat, Sprague-Dawley (M, F)	Bone marrow	Chromosomal damage	Chromosomal aberrations	–	1000 mg/kg bw	i.p.; 1 ×; sampled after 6, 12 and 24 h	Single dose tested only	Li & Long (1988)
Mouse, NMRI-bom (M, F)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	–	200 mg/kg bw	i.p.; 1 ×; sampled after 24 and 48 h	Glyphosate isopropylamine salt	Rank *et al.* (1993)
Mouse, Swiss CD1 (M)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	+	300 mg/kg bw	i.p.; 2 × 150 mg/kg bw with 24 h interval; sampled 6 or 24 h after the last injection	Single dose tested only $P < 0.05$ after 24 h	Bolognesi *et al.* (1997)

Table 4.3 (continued)

Species, strain (sex)	Tissue	End-point	Test	Results	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Mouse, Balb C (M, F)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	+	400 mg/kg bw	i.p.; one injection per 24 h, 2 × 200, sampled 24 h after the last injection	$P < 0.01$ at the highest dose (400 mg/kg bw)	Mañas *et al.* (2009a)
AMPA								
Mouse, Balb C (M, F)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	+	200 mg/kg bw	i.p.; one injection per 24 h, 2 × 100, sampled 24 h after the last injection	$P < 0.01$ at the lowest dose (200 mg/kg bw)	Mañas *et al.* (2009b)
Glyphosate-based formulations								
Mouse, Swiss CD1 (M)	Liver	DNA damage	DNA adducts, 8-OHdG by LC/UV	–	~300 mg/kg bw	i.p.; 1 ×, sampled after 8 and 24 h	Glyphosate, 30.4% Single dose tested only	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M)	Kidney	DNA damage	DNA adducts, 8-OHdG by LC/UV	+	~300 mg/kg bw	i.p.; 1 ×, sampled after 8 and 24 h	Glyphosate, 30.4% Single dose tested only $P < 0.05$	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M, F)	Kidney	DNA damage	DNA adducts, ^{32}P-DNA post labelling	+	400 mg/kg bw	i.p.; 1 ×; sampled after 24 h	Glyphosate isopropylammonium salt, 30.4%	Peluso *et al.* (1998)
Mouse, Swiss CD1 (M, F)	Liver	DNA damage	DNA adducts, ^{32}P-DNA post labelling	+	400 mg/kg bw	i.p.; 1 ×; sampled after 24 h	Glyphosate isopropylammonium salt, 30.4%	Peluso *et al.* (1998)
Mouse, Swiss CD1 (M)	Liver	DNA damage	DNA strand breaks, alkaline elution assay	+	~300 mg/kg bw	i.p.; 1 ×; sampled after 4 and 24 h	Glyphosate, 30.4% Single dose tested only $P < 0.05$ only after 4 h	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M)	Kidney	DNA damage	DNA strand breaks, alkaline elution assay	+	~300 mg/kg bw	i.p.; 1 ×; sampled after 4 and 24 h	Glyphosate, 30.4% Single dose tested only $P < 0.05$ only after 4 h	Bolognesi *et al.* (1997)
Mouse, C57BL (M)	Bone marrow (PCE)	Chromosomal damage	Chromosomal aberrations	–	1080 mg/kg bw	p.o. in distilled water; 1 ×; sampled after 6, 24, 48, 72, 96 and 120 h	Single dose tested only	Dimitrov *et al.* (2006)

Table 4.3 (continued)

Species, strain (sex)	Tissue	End-point	Test	Results	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Mouse, Swiss albino (M)	Bone marrow	Chromosomal damage	Chromosomal aberrations	+	25 mg/kg bw	i.p.; 1 ×; sampled after 24, 48 and 72 h	Glyphosate isopropylamine salt, > 41% The percentage of aberrant cells was increased vs control in a dose- and time-dependent manner ($P < 0.05$)	Prasad *et al.* (2009)
Mouse, NMRI-bom (M, F)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	–	200 mg/kg bw	i.p.; 1 ×; sampled after 24 h	Glyphosate isopropylammonium salt, 480 g/L The percentage of PCE decreased	Rank *et al.* (1993)
Mouse, Swiss (M, F)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	–	200 mg/kg bw	i.p.; 2 × within 24 h interval and sampled 24 h after the last injection	Glyphosate isopropylammonium salt, 480 g/L	Grisolia (2002)
Mouse, Swiss albino (M)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	+	25 mg/kg bw	i.p.; 1 × ; sampled after 24, 48 and 72 h	Glyphosate isopropylamine salt, > 41% Significant induction of micronuclei vs control at both doses and all times ($P < 0.05$)	Prasad *et al.* (2009)
Mouse, Swiss CD1 (M)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	+	450 mg/kg bw	i.p.; 2 × 225 mg/kg with 24 h interval; sampled 6 or 24 h after the last injection	Glyphosate, 30.4% Single dose tested only $P < 0.05$ after 6 h and 24 h	Bolognesi *et al.* (1997)
Mouse, C57BL (M)	Bone marrow	Chromosomal damage	Micronucleus formation	–	1080 mg/kg bw	p.o. in distilled water; 1 × ; sampled after 24, 48, 72, 96 and 120 h	Single dose tested only	Dimitrov *et al.* (2006)

[a] +, positive; -, negative; (+) or (-) positive/negative in a study with limited quality

bw, body weight; F, female; h, hour; HID, highest effective dose; i.p., intraperitoneal; LC, liquid chromatography; LED, lowest effective dose; M, male; PCE, polychromatic erythrocytes; p.o., oral; 8-OHdG, 8-hydroxydeoxyguanosine; UV, ultraviolet

Table 4.4 Genetic and related effects of glyphosate, AMPA, and glyphosate-based formulations in non-human mammalian cells in vitro

Species	Tissue, cell line	End-point	Test	Results[a]		Dose (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Glyphosate								
Rat, Fisher F334	Hepatocytes	DNA damage	Unscheduled DNA synthesis	–	NT	125 µg/mL		Li & Long (1988)
Hamster, Chinese	CHO-K_1BH_1 ovary, cell line	Mutation	*Hprt* mutation	–	–	22 500 µg/mL		Li & Long (1988)
Bovine	Lymphocytes	Chromosomal damage	Chromosomal aberrations	+	NT	17 µM [3 µg/mL]	$P < 0.05$	Lioi *et al.* (1998)
Hamster, Chinese	CHO-K1 ovary cell line	Chromosomal damage	Micronucleus formation	–	+	10 µg/mL	$P \leq 0.001$, in the dark +S9 Negative –S9 in the dark or with light irradiation	Roustan *et al.* (2014)
Bovine	Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	17 µM [3 µg/mL]	$P < 0.05$	Lioi *et al.* (1998)
AMPA								
Hamster, Chinese	CHO-K1 ovary cell line	Chromosomal damage	Micronucleus formation	+	+	0.01 µg/mL	$P \leq 0.05$, in the dark –S9 Highest increase was observed at very low dose (0.0005 µg/mL) –S9 but with light-irradiation ($P < 0.01$)	Roustan *et al.* (2014)
Glyphosate-based formulations								
Bovine	Lymphocytes	Chromosomal damage	Chromosomal aberrations	–	NT	1120 µM [190 µg/mL]	Glyphosate, 62%	Siviková & Dianovský (2006)
Bovine	Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	–	56 µM [9.5 µg/mL]	Glyphosate, 62% Time of exposure, 24 h $P < 0.01$, –S9, at ≥ 56 µM	Siviková & Dianovský (2006)

[a] +, positive; –, negative; (+), weakly positive

AMPA, aminomethyl phosphonic acid; HIC, highest ineffective concentration; *Hprt*, hypoxanthine guanine phosphoribosyl transferase gene; LEC, lowest effective concentration; NT, not tested

Table 4.5 Genetic and related effects of glyphosate, AMPA, and glyphosate-based formulations in non-mammalian systems in vivo

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Glyphosate							
Fish	*Prochilodus lineatus* (sábalo), erythrocytes and gill cells	DNA damage	DNA strand breaks, comet assay	+	0.48 mg/L	Time of exposure 6, 24, and 96 h For erythrocytes, $P = 0.01$ after 6 h, and $P = 0.014$ after 96 h; no significant increase after 24 h For gill cells, $P = 0.02$ only after 6 h at 2.4 mg/L	Moreno *et al.* (2014)
Fish	*Anguilla anguilla* L. (European eel), blood cells	DNA damage	DNA strand breaks, comet assay	+	0.0179 mg/L	Time of exposure 1 and 3 days $P < 0.05$	Guilherme *et al.* (2012b)
Fish	*Danio rerio* (zebrafish), sperm	DNA damage	DNA strand breaks, acridine orange method	+	10 mg/L	After 96 h, DNA integrity was 78.3 ± 3.5%, significantly reduced from control (94.7 ± 0.9%) and 5 mg/L (92.6 ± 1.9%), ($P < 0.05$)	Lopes *et al.* (2014)
Fish	*Oreochromis niloticus* (Nile tilapia) branchial erythrocytes	DNA damage	DNA strand breaks, comet assay	+	7 µM [1.2 mg/L]	Time of exposure, 10 days $P < 0.001$ with concentrations ≥ 7 µM	Alvarez-Moya *et al.* (2014)
Oyster	Oyster spermatozoa	DNA damage	DNA strand breaks, comet assay	–	0.005 mg/L	Time of exposure, 1 h	Akcha *et al.* (2012)
Insect	*Drosophila* standard cross	Mutation	SMART	+	1 mM [0.169 mg/L]	Purity, 96% Increased frequency of small single spots (≥ 1 mM) and total spots (≥ 2 mM) $P = 0.05$	Kaya *et al.* (2000)
Insect	*Drosophila melanogaster*, high bioactivation cross	Mutation	SMART	–	10 mM [1.69 mg/L]	Purity, 96%	Kaya *et al.* (2000)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Plant systems	*Tradescantia* clone 4430 (spiderworts), staminal hair nuclei	DNA damage	DNA strand breaks, comet assay	+	0.0007 mM [0.12 µg/mL]	Glyphosate isopropylamine salt $P < 0.01$ for directly exposed nuclei (dose-dependent increase) and plants	Alvarez-Moya *et al.* (2011)
Plant systems	*Allium cepa* (onion)	Chromosomal damage	Chromosomal aberrations	+	3%	Single dose tested only Partial but significant reversal with distilled water	Frescura *et al.* (2013)
Plant systems	*Allium cepa* (onion)	Chromosomal damage	Chromosomal aberrations	–	2.88 µg/mL	Glyphosate isopropylamine	Rank *et al.* (1993)
Plant systems	*Trigonella foenum-graecum* L. (fenugreek)	Chromosomal damage	Chromosomal aberrations	+	0.2%	$P < 0.001$; positive dose–response relationship	Siddiqui *et al.* (2012)
Plant systems	*Vicia faba* (bean)	Chromosomal damage	Micronucleus formation	–	1400 ppm (1400 µg/g of soil)	Tested with two types of soil, but not without soil	De Marco *et al.* (1992)
AMPA							
Fish	*Anguilla anguilla* L. (European eel)	DNA damage	DNA strand breaks, comet assay	+	0.0118 mg/L	Time of exposure, 1 and 3 days $P < 0.05$ after 1 day of exposure	Guilherme *et al.* (2014b)
Fish	*Anguilla anguilla* L. (European eel)	Chromosomal damage	Other (ENA)	+	0.0236 mg/L	$P < 0.05$ only at highest dose after 3 day exposure (not after 1 day)	Guilherme *et al.* (2014b)
Glyphosate-based formulations							
Fish	*Anguilla anguilla* L. (European eel), blood cells	DNA damage	DNA strand breaks, comet assay	+	0.058 mg/L	$P < 0.05$ Positive dose–response relationship	Guilherme *et al.* (2010)
Fish	*Anguilla anguilla* L. (European eel), blood cells	DNA damage	DNA strand breaks, comet assay improved with the DNA-lesion-specific FPG and Endo III	+	0.058 mg/L	Glyphosate-based formulation, 30.8% Time of exposure, 1 and 3 days With FPG, $P < 0.05$; with comet assay alone, $P < 0.05$ at 116 µg/L	Guilherme *et al.* (2012b)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Fish	*Anguilla anguilla* L. (European eel), blood cells	DNA damage	DNA strand breaks, comet assay improved with the DNA-lesion-specific FPG and Endo III	+	0.116 mg/L	Single dose tested only Time of exposure, 3 days; recovery from non-specific DNA damage, but not oxidative DNA damage, 14 days after exposure $P < 0.05$	Guilherme *et al.* (2014a)
Fish	*Anguilla anguilla* L. (European eel), liver	DNA damage	DNA strand breaks, comet assay improved with the DNA-lesion-specific FPG and Endo III	+	0.058 mg/L	Glyphosate-based formulation, 485 g/L Time of exposure, 3 days $P < 0.05$	Marques *et al.* (2014, 2015)
Fish	*Prochilodus lineatus* (sábalo), erythrocytes and bronchial cells	DNA damage	DNA strand breaks, comet assay	+	10 mg/L	Single dose tested only, for 6, 24, and 96 h $P < 0.05$ for both erythrocytes and bronchial cells	Cavalcante *et al.* (2008)
Fish	*Prochilodus lineatus* (sábalo), erythrocytes and gill cells	DNA damage	DNA strand breaks, comet assay	+	1 mg/L	Glyphosate-based formulation, 480 g/L Time of exposure, 6, 24 and 96 h $P < 0.001$ after 24 and 96 h in erythrocytes and 24 h in gill cells	Moreno *et al.* (2014)
Fish	*Poecilia reticulata* (guppy) gill erythrocytes	DNA damage	DNA strand breaks, comet assay	+	2.83 µL/L [1.833 mg/L]	Glyphosate, 64.8%, m/v (648 g/L) $P < 0.05$	De Souza Filho *et al.* (2013)
Fish	*Channa punctatus* (bloch), blood and gill cells	DNA damage	DNA strand breaks, comet assay	+	3.25 mg/L	Exposure continued for 35 days; blood and gill cells collected on day 1, 7, 14, 21, 28 and 35 $P < 0.01$, for blood and gill cells; DNA damage increased with time and concentration	Nwani *et al.* (2013)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Fish	*Corydoras paleatus* (blue leopard corydoras, mottled corydoras and peppered catfish), blood and hepatic cells	DNA damage	DNA strand breaks, comet assay	+	0.0067 mg/L	Glyphosate, 48% (corresponding to 3.20 µg/L) Single dose tested only, for 3, 6, and 9 days $P < 0.01$, in blood and in liver cells	de Castilhos Ghisi & Cestari (2013)
Fish	*Cyprinus carpio* Linnaeus (carp), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	2 mg/L (10% LC_{50}, 96 h)	Glyphosate, equivalent to 360 g/L Single dose tested only, for 16 days $P < 0.01$	Gholami-Seyedkolaei *et al.* (2013)
Fish	*Carassius auratus* (goldfish), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	5 ppm	Glyphosate equivalent to 360 g/L Time of exposure, 2, 4 and 6 days After 48 h: $P < 0.05$ (5 mg/L) and $P < 0.001$ (10 and 15 mg/L)	Cavas & Könen (2007)
Fish	*Prochilodus lineatus* (sábalo) erythrocytes	Chromosomal damage	Micronucleus formation	–	10 mg/L	Single dose tested only, for 6, 24, and 96 h Nuclear abnormalities (lobed nuclei, segmented nuclei and kidney-shaped nuclei)	Cavalcante *et al.* (2008)
Fish	*Corydoras paleatus* (blue leopard corydoras, mottled corydoras and peppered catfish), blood and hepatic cells	Chromosomal damage	Micronucleus formation	–	0.0067 mg/L	Glyphosate, 48% (corresponding to 3.20 µg/L) Single dose tested only, for 3, 6 and 9 days	de Castilhos Ghisi & Cestari (2013)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Fish	*Tilapia rendalli* (redbreast tilapia) blood erythrocytes	Chromosomal damage	Micronucleus formation	+	42 mg/kg bw	Glyphosate, 480 g/L Increased frequency of micronucleus formation vs control ($P < 0.05$) in blood samples collected 4 days after a single intra-abdominal injection of 42, 85, or 170 mg/kg bw	Grisolia (2002)
Fish	*Carassius auratus* (goldfish), erythrocytes	Chromosomal damage	Micronucleus formation	+	5 ppm	Glyphosate equivalent to 360 g/L Time of exposure, 2, 4 and 6 days Statistically significant differences: 96 h ($P < 0.05$); 144 h ($P < 0.01$)	Cavas & Könen (2007)
Fish	*Poecilia reticulata* (guppy) gill erythrocytes	Chromosomal damage	Micronucleus formation, ENA	+	1.41 µL/L [0.914 mg/L]	Glyphosate, 64.8%, m/v (648 g/L) Micronucleus formation, $P < 0.01$ Other nuclear abnormalities, $P < 0.05$ at 1.41 to 5.65 µL/L; concentration-dependent ($r^2 = 0.99$)	De Souza Filho *et al.* (2013)
Fish	*Cnesterodon decemmaculatus* (Jenyns, 1842) peripheral blood erythrocytes	Chromosomal damage	Micronucleus formation	+	3.9 mg/L	Glyphosate, 48% Time of exposure, 48 and 96 h $P < 0.05$, with 3.9 and 7.8 mg/L for 48 and 96 h	Vera-Candioti *et al.* (2013)
Fish	*Cnesterodon decemmaculatus* (Jenyns, 1842) peripheral blood erythrocytes	Chromosomal damage	Micronucleus formation	+	22.9 mg/L	Glyphosate, 48% Time of exposure, 48 and 96 h $P < 0.01$, with 22.9 and 45.9 mg/L, and $P < 0.05$ at 68.8 mg/L, for 96 h	Vera-Candioti *et al.* (2013)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Fish	*Prochilodus lineatus* (sábalo) erythrocytes	Chromosomal damage	Chromosomal aberrations	–	10 mg/L	Single dose tested only, for 6, 24, and 96 h Nuclear abnormalities (lobed nuclei, segmented nuclei and kidney-shaped nuclei)	Cavalcante *et al.* (2008)
Fish	*Anguilla anguilla* L. (European eel), peripheral mature erythrocytes	Chromosomal damage	Other (ENA)	+	0.058 mg/L	Time of exposure, 1 and 3 days Chromosomal breakage and/or chromosomal segregational abnormalities after 3 days of exposure, $P < 0.05$	Guilherme *et al.* (2010)
Caiman	*Caiman latirostris* (broad-snouted caiman), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	0.500 mg/egg	Glyphosate, 66.2% In-ovo exposure; blood sampling at the time of hatching $P < 0.05$ in both experiments (50–1000 µg/egg in experiment 1; 500–1750 µg/egg in experiment 2)	Poletta *et al.* (2009)
Caiman	*Caiman latirostris* (broad-snouted caiman), erythrocytes	DNA damage	DNA strand breaks, comet assay	–	19 800 mg/L	Glyphosate, 66.2% Single dose tested only; in-ovo exposure First spraying exposure at the beginning of incubation period, a second exposure on day 35, then incubation until hatching	Poletta *et al.* (2011)
Caiman	*Caiman latirostris* (broad-snouted caiman), erythrocytes	Chromosomal damage	Micronucleus fomation	+	0.500 mg/egg	Glyphosate, 66.2% In-ovo exposure; blood sampling at the time of hatching $P < 0.05$ in both experiments (50–1000 µg/egg in experiment 1; 500–1750 µg/egg in experiment 2)	Poletta *et al.* (2009)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Caiman	*Caiman latirostris* (broad-snouted caiman), erythrocytes	Chromosomal damage	Micronucleus fomation	+	19.8 g/L	Glyphosate, 66.2% One dose tested; in-ovo exposure First spraying exposure at the beginning of incubation period, a second exposure on day 35, then incubation until hatching. Micronucleus formation, $P < 0.001$ Damage index, $P < 0.001$	Poletta *et al.* (2011)
Frog tadpole	*Rana catesbeiana* (ouaouaron), blood	DNA damage	DNA strand breaks, comet assay	+	1.687 mg/L, p.o.	Time of exposure, 24 h $P < 0.05$, with 6.75 mg/L; and $P < 0.001$ with 27 mg/L (with 108 mg/L, all died within 24 h)	Clements *et al.* (1997)
Frog	*Eleutherodactylus johnstonei* (Antilles coqui), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	0.5 µg a.e./cm²	Glyphosate-based formulation, 480 g/L Exposure to an homogenate mist in a 300 cm² glass terrarium Time of exposure: 0.5, 1, 2, 4, 8 and 24 h $P < 0.05$	Meza-Joya *et al.* (2013)
Frog	*Euflictis cyanophlyctis* (Indian skittering frog), erythrocytes	Chromosomal damage	Micronucleus formation	+	1 mg a.e./L	Glyphosate isopropylamine salt, 41% Time of exposure: 24, 48, 72, and 96 h $P < 0.001$ at 24, 48, 72 and 96 h	Yadav *et al.* (2013)
Snail	*Biomphalaria alexandrina*, haemolymph	DNA damage	DNA strand breaks, comet assay	+	10 mg/L	Glyphosate, 48% Single dose tested only, for 24 h. The percentage of damaged DNA was 21% vs 4% (control) No statistical analysis	Mohamed (2011)
Oyster	Oysters, spermatozoa	DNA damage	DNA strand breaks, comet assay	–	5 µg/L	Glyphosate, 200 µg equivalent/L Time of exposure, 1 h	Akcha *et al.* (2012)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Clam	*Corbicula fluminea* (Asian clam) haemocytes	DNA damage	DNA strand breaks, comet assay	–	10 mg/L	Time of exposure, 96 h Significant increase when atrazine (2 or 10 mg/L) was added to glyphosate ($P < 0.05$) No increase after exposure to atrazine or glyphosate separately	dos Santos & Martinez (2014)
Mussels	*Utterbackia imbecillis* (Bivalvia: Unionidae) glochidia mussels (larvae)	DNA damage	DNA strand breaks, comet assay	–	5 mg/L	Glyphosate, 18% Doses tested: 2.5 and 5 mg/L for 24 h NOEC, 10.04 mg/L	Conners & Black (2004)
Worm	Earthworm, *Eisenia andrei*, coelomocytes	DNA damage	DNA strand breaks, comet assay	–	240 µg a.e./cm²	Monoammonium salt, 85.4%, a.e. Epidermic exposure during 72 h (on filter paper)	Piola *et al.* (2013)
Worm	Earthworm, *Eisenia andrei*, coelomocytes	DNA damage	DNA strand breaks, comet assay	+	15 µg a.e./cm²	Monoammonium salt, 72%, a.e. Epidermic exposure during 72 h (on filter paper) $P < 0.001$	Piola *et al.* (2013)
Worm	Earthworm, *Pheretima peguana*, coelomocytes	DNA damage	DNA strand breaks, comet assay	–	251.50 µg/cm²	Active ingredient, 36% (w/v) Epidermic exposure 48 h on filter paper; LC_{50}, 251.50 µg/cm²	Muangphra *et al.* (2014)
Worm	Earthworm, *Pheretima peguana*, coelomocytes	Chromosomal damage	Micronucleus formation	+	251.50 µg/cm²	Active ingredient, 36% (w/v) Exposure, 48 h on filter paper; LC_{50}, 251.50 µg/cm² filter paper $P < 0.05$, for total micro-, bi-, and trinuclei frequencies at 0.25 µg/cm²; when analysed separately, micro- and trinuclei frequencies significantly differed from controls only at the LC_{50}	Muangphra *et al.* (2014)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Insect	*Drosophila melanogaster*	Mutation	Sex-linked recessive lethal mutations	+	1 ppm	Single dose tested only $P < 0.001$	Kale *et al.* (1995)
Plant systems	*Allium cepa* (onion)	Chromosomal damage	Chromosomal aberrations	+	1.44 µg/mL	Glyphosate-based formulation, 480 g/L The doses of formulation were calculated as glyphosate isopropylamine $P < 0.005$	Rank *et al.* (1993)
Plant systems	*Crepis capillaris* (hawksbeard)	Chromosomal damage	Chromosomal aberrations	–	0.5%	The highest dose tested (1%) was toxic	Dimitrov *et al.* (2006)
Plant systems	*Hordeum vulgare* L. cv. Madalin (barley roots)	Chromosomal damage	Chromosomal aberrations	(+)	360 µg/mL (0.1%)	Reported as "significant"	Truta *et al.* (2011)
Plant systems	*Crepis capillaris* (hawksbeard)	Chromosomal damage	Micronucleus formation	–	0.5%	The highest dose tested (1%) was toxic	Dimitrov *et al.* (2006)

[a] +, positive; –, negative; (+) or (–) positive/negative in a study with limited quality

a.e., acid equivalent; AMPA, aminomethyl phosphonic acid; bw, body weight; ENA, erythrocytic nuclear abnormalities; Endo III, endonuclease III; FPG, formamidopyrimidine glycosylase; h, hour; HID, highest ineffective dose; LC_{50}, median lethal dose; LED, lowest effective dose; NOEC, no-observed effect concentration; p.o., oral; SMART, somatic mutation and recombination test

Table 4.6 Genetic and related effects of glyphosate and glyphosate-based formulations on non-mammalian systems in vitro

Phylogenetic class	Test system (species; strain)	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Glyphosate								
Eukaryote Fish	*Oreochromis niloticus* (Nile tilapia), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	NT	7 µM [1.2 µg/mL]	Glyphosate isopropylamine, 96% $P \leq 0.001$; positive dose–response relationship for doses ≥ 7 µM	Alvarez-Moya *et al.* (2014)
Prokaryote (bacteria)	*Scytonema javanicum* (cyanobacteria)	DNA damage	DNA strand breaks, FADU assay	(+)	NT	10 µM [1.7 µg/mL] (in combination with UVB)	Co-exposure to glyphosate (not tested alone; single dose tested only) enhanced UVB-induced increases	Wang *et al.* (2012)
Prokaryote (bacteria)	*Anabaena spherica* (cyanobacteria)	DNA damage	DNA strand breaks, FADU assay	(+)	NT	10 µM [1.7 µg/mL] (in combination with UVB)	Co-exposure to glyphosate (not tested alone; single dose tested only) enhanced UVB-induced increases	Chen *et al.* (2012)
Prokaryote (bacteria)	*Microcystis viridis* (cyanobacteria)	DNA damage	DNA strand breaks, FADU assay	(+)	NT	10 µM [1.7 µg/mL] (in combination with UVB)	Co-exposure to glyphosate (not tested alone; single dose tested only) enhanced UVB-induced increases	Chen *et al.* (2012)
Prokaryote (bacteria)	*Bacillus B. subtilis*	Differential toxicity	Rec assay	–	NT	2000 µg/disk		Li & Long (1988)
Prokaryote (bacteria)	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA98 and TA100	Mutation	Reverse mutation	–	–	5000 µg/plate		Li & Long (1988)
Prokaryote (bacteria)	*Escherichia coli* WP2	Mutation	Reverse mutation	–	–	5000 µg/plate		Li & Long (1988)

Table 4.6 (continued)

Phylogenetic class	Test system (species; strain)	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Acellular systems	Prophage superhelical PM2 DNA	DNA damage	DNA strand breaks	(–)	NT	75 mM [12.7 mg/mL] (in combination with H_2O_2 (100 µM)	Glyphosate inhibited H_2O_2-induced damage of PM2 DNA at concentrations where synergism was observed in cellular DNA damage (data NR)	Lucken *et al.* (2004)
Glyphosate-based formulations								
Prokaryote (bacteria)	*Salmonella typhimurium* TA98	Mutation	Reverse mutation	+	–	360 µg/plate	Glyphosate isopropylammonium salt, 480 g/L	Rank *et al.* (1993)
Prokaryote (bacteria)	*Salmonella typhimirium* TA100	Mutation	Reverse mutation	–	+	720 µg/plate	Glyphosate isopropylammonium salt, 480 g/L	Rank *et al.* (1993)

[a] +, positive; –, negative; (+) or (–) positive/negative in a study with limited quality

FADU, fluorometric analysis of DNA unwinding; HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested; UVB, ultraviolet B

Additionally, although all four glyphosate-based formulations dramatically reduced the transcription of ERα and ERβ in ERE-transfected HepG2 cells, glyphosate alone had no significant effect. Glyphosate and all four formulations reduced androgen-receptor transcription in the breast cancer cell line MDA-MB453-kb2, which has a high level of androgen receptor, with the formulations showing greater activity than glyphosate alone.

In a human placental cell line derived from choriocarcinoma (JEG3 cells), 18 hours of exposure to a glyphosate-based formulation (IC_{50} = 0.04%) decreased aromatase activity (Richard *et al.*, 2005). Glyphosate alone was without effect. The concentrations used did not affect cell viability.

Glyphosate, at non-overtly toxic concentrations, decreased aromatase activity in fresh human placental microsomes and transformed human embryonic kidney cells (293) transfected with human aromatase cDNA (Benachour *et al.*, 2007). A glyphosate-based formulation, at non-overtly toxic concentrations, had the same effect. The formulation was more active at equivalent doses than glyphosate alone.

In human androgen receptor and ERα and ERβ reporter gene assays using the Chinese hamster ovary cell line (CHO-K1), glyphosate had neither agonist nor antagonist activity (Kojima *et al.*, 2004, 2010).

(ii) Non-human mammalian experimental systems

In vivo

No data were available to the Working Group.

In vitro

Benachour *et al.* (2007) and Richard *et al.* (2005) reported that glyphosate and a glyphosate-based formulation inhibited aromatase activity in microsomes derived from equine testis. Richard *et al.* (2005) reported an absorbance spectrum consistent with an interaction between a nitrogen atom of glyphosate and the active site of the purified equine aromatase enzyme.

In the mouse MA-10 Leydig cell tumour cell line, a glyphosate-based formulation (glyphosate, 180 mg/L) markedly reduced [$(Bu)_2$] cAMP-stimulated progesterone production (Walsh *et al.*, 2000). The inhibition was dose-dependent, and occurred in the absence of toxicity or parallel reductions in total protein synthesis. In companion studies, the formulation also disrupted steroidogenic acute regulatory protein expression, which is critical for steroid hormone synthesis. Glyphosate alone did not affect steroidogenesis at any dose tested up to 100 µg/L. Forgacs *et al.* (2012) found that glyphosate (300 µM) had no effect on testosterone production in a novel murine Leydig cell line (BLTK1). Glyphosate did not modulate the effect of recombinant human chorionic gonadotropin, which served as the positive control for testosterone production.

(iii) Non-mammalian experimental systems

Gonadal tissue levels of testosterone, 17β-estradiol and total microsomal protein were significantly reduced in adult snails (*Biomphalaria alexandrina*) exposed for 3 weeks to a glyphosate-based formulation (glyphosate, 48%) at the LC_{10} (10% lethal concentration) (Omran & Salama, 2013). These effects persisted after a 2-week recovery period, although the impact on 17β-estradiol was reduced in the recovery animals. The formulation also induced marked degenerative changes in the ovotestis, including absence of almost all the gametogenesis stages. CYP450 1B1, measured by enzyme-linked immunosorbent assay (ELISA), was substantially increased in the treated snails, including after the recovery period.

Glyphosate (0.11 mg/L for 7 days) did not increase plasma vittelogenin levels in juvenile rainbow trout (Xie *et al.*, 2005).

(b) Other pathways

(i) Humans

Studies in exposed humans

No data were available to the Working Group.

Human cells in vitro

Glyphosate did not exhibit agonist activity in an assay for a human pregnane X receptor (PXR) reporter gene in a CHO-K1 cell line (Kojima *et al.*, 2010).

(ii) Non-human mammalian experimental systems

In vivo

In rats, glyphosate (300 mg/kg bw, 5 days per week, for 2 weeks) had no effect on the formation of peroxisomes, or the activity of hepatic carnitine acetyltransferase and catalase, and did not cause hypolipidaemia, suggesting that glyphosate does not have peroxisome proliferator-activated receptor activity (Vainio *et al.*, 1983).

In vitro

Glyphosate was not an agonist for mouse peroxisome proliferator-activated receptors PPARα or PPARγ in reporter gene assays using CV-1 monkey kidney cells in vitro (Kojima *et al.*, 2010). Glyphosate was also not an agonist for the aryl hydrocarbon receptor in mouse hepatoma Hepa1c1c7 cells stably transfected with a reporter plasmid containing copies of dioxin-responsive element (Takeuchi *et al.*, 2008).

(iii) Non-mammalian experimental systems

As a follow-up to experiments in which injection of glyphosate, or incubation with a glyphosate-based formulation (glyphosate, 48%), caused chick and frog (*Xenopus laevis*) cephalic and neural crest terata characteristic of retinoic acid signalling dysfunction, Paganelli *et al.*, (2010) measured retinoic acid activity in tadpoles exposed to a glyphosate-based formulation. Retinoic activity measured by a reporter gene assay was increased by the formulation, and a retinoic acid antagonist blocked the effect. This indicated a possible significant modulation of retinoic acid activity by glyphosate.

4.2.3 Oxidative stress, inflammation, and immunosuppression

(a) Oxidative stress

(i) Humans

Studies in exposed humans

No data were available to the Working Group.

Human cells in vitro

Several studies examined the effects of glyphosate on oxidative stress parameters in the human keratinocyte cell line HaCaT. Gehin *et al.* (2005) found that a glyphosate-based formulation was cytotoxic to HaCaT cells, but that addition of antioxidants reduced cytotoxicity. Elie-Caille *et al.* (2010) showed that incubation of HaCaT cells with glyphosate at 21 mM (the half maximal inhibitory concentration for cytotoxicity, IC_{50}) for 18 hours increased production of hydrogen peroxide (H_2O_2) as shown by dichlorodihydrofluorescein diacetate assay. Similarly, George & Shukla (2013) exposed HaCaT cells to a glyphosate-based formulation (glyphosate, 41%; concentration, up to 0.1 mM) and evaluated oxidative stress using the dichlorodihydrofluorescein diacetate assay. The formulation (0.1 mM) increased maximum oxidant levels by approximately 90% compared with vehicle, an effect similar to that of H_2O_2 (100 mM). Pre-treatment of the cells with the antioxidant *N*-acetylcysteine abrogated generation of oxidants by both the formulation and by H_2O_2. *N*-Acetylcysteine also inhibited cell proliferation induced by the glyphosate-based formulation (0.1 mM). [The Working Group noted the recognized limitations of using dichlorodihydrofluorescein diacetate as a marker of oxidative stress (Bonini *et al.*, 2006; Kalyanaraman *et al.*, 2012),

and that the studies that reported this end-point as the sole evidence for oxidative stress should thus be interpreted with caution.]

Chaufan *et al.* (2014) evaluated the effects of glyphosate, AMPA (the main metabolite of glyphosate), and a glyphosate-based formulation on oxidative stress in HepG2 cells. The formulation, but not glyphosate or AMPA, had adverse effects. Specifically, the formulation increased levels of reactive oxygen species, nitrotyrosine formation, superoxide dismutase activity, and glutathione, but did not have an effect on catalase or glutathione-*S*-transferase activities. Coalova *et al.* (2014) exposed Hep2 cells to a glyphosate-based formulation (glyphosate as isopropylamine salt, 48%) at the LC_{20} (concentration not otherwise specified) and evaluated various parameters of oxidative stress. Exposure to the formulation for 24 hours increased catalase activity and glutathione levels, but did not have an effect on superoxide dismutase or glutathione-*S*-transferase activity.

Using blood samples from non-smoking male donors, Mladinic *et al.* (2009b) examined the effects of in-vitro exposure to glyphosate on oxidative DNA damage in primary lymphocyte cultures and on lipid peroxidation in plasma. Both parameters were significantly elevated at glyphosate concentrations of 580 µg/mL (~3.4 mM), but not at lower concentrations. Kwiatkowska *et al.* (2014) examined the effects of glyphosate, its metabolite AMPA, and *N*-methylglyphosate (among other related compounds) in human erythrocytes isolated from healthy donors. The erythrocytes were exposed at concentrations of 0.01–5 mM for 1, 4, or 24 hours before flow cytometric measurement of the production of reactive oxygen species with dihydrorhodamine 123. Production of reactive oxygen species was increased by glyphosate (≥ 0.25 mM), AMPA (≥ 0.25 mM), and *N*-methylglyphosate (≥ 0.5 mM).

(ii) Non-human mammalian experimental systems

Most of the studies of oxidative stress and glyphosate were conducted in rats and mice, and examined a range of exposure durations, doses, preparations (glyphosate and glyphosate-based formulations), administration routes and tissues. In addition, various end-points were evaluated to determine whether oxidative stress is induced by exposure to glyphosate. Specifically, it was found that glyphosate induces production of free radicals and oxidative stress in mouse and rat tissues through alteration of antioxidant enzyme activity, depletion of glutathione, and increases in lipid peroxidation. Increases in biomarkers of oxidative stress upon exposure to glyphosate in vivo have been observed in blood plasma (Astiz *et al.*, 2009b), liver (Bolognesi *et al.*, 1997; Astiz *et al.*, 2009b), skin (George *et al.*, 2010), kidney (Bolognesi *et al.*, 1997; Astiz *et al.*, 2009b), and brain (Astiz *et al.*, 2009b). Several studies demonstrated similar effects with a glyphosate-based formulation in the liver (Bolognesi *et al.*, 1997; Cavuşoğlu *et al.*, 2011; Jasper *et al.*, 2012), kidney (Bolognesi *et al.*, 1997; Cavuşoğlu *et al.*, 2011) and brain (Cattani *et al.*, 2014), or with a pesticide mixture containing glyphosate in the testes (Astiz *et al.*, 2013). Pre-treatment with antioxidants has been shown to mitigate the induction of oxidative stress by a glyphosate-based formulation (Cavuşoğlu *et al.*, 2011) and by a pesticide mixture containing glyphosate (Astiz *et al.*, 2013).

DNA damage associated with oxidative stress after exposure to glyphosate (e.g. as reported in Bolognesi *et al.*, 1997) is reviewed in Section 4.2.1.

(iii) Non-mammalian experimental systems

Positive associations between exposure to glyphosate and oxidative stress were reported in various tissues in aquatic organisms (reviewed in Slaninova *et al.*, 2009). Glyphosate and various glyphosate-based formulations have been tested in various fish species for effects on a plethora of end-points (e.g. lipid peroxidation, DNA

damage, expression of antioxidant enzymes, levels of glutathione), consistently presenting evidence that glyphosate can cause oxidative stress in fish (Lushchak *et al.*, 2009; Ferreira *et al.*, 2010; Guilherme *et al.*, 2010, 2012a, b, 2014a, b; Modesto & Martinez, 2010a, b; Cattaneo *et al.*, 2011; Glusczak *et al.*, 2011; de Menezes *et al.*, 2011; Ortiz-Ordoñez *et al.*, 2011; Nwani *et al.*, 2013; Marques *et al.*, 2014, 2015; Sinhorin *et al.*, 2014; Uren Webster *et al.*, 2014). Similar effects were observed in bullfrog tadpoles exposed to a glyphosate-based formulation (Costa *et al.*, 2008), and in the Pacific oyster exposed to a pesticide mixture containing glyphosate (Geret *et al.*, 2013).

(b) Inflammation and immunomodulation

(i) Humans

Studies in exposed humans

No data were available to the Working Group.

Human cells in vitro

Nakashima *et al.* (2002) investigated the effects of glyphosate on cytokine production in human peripheral blood mononuclear cells. Glyphosate (1 mM) had a slight inhibitory effect on cell proliferation, and modestly inhibited the production of IFN-gamma and IL-2. The production of TNF-α and IL-1 β was not affected by glyphosate at concentrations that significantly inhibited proliferative activity and T-cell-derived cytokine production.

(ii) Non-human mammalian experimental systems

Kumar *et al.* (2014) studied the pro-inflammatory effects of glyphosate and farm air samples in wildtype C57BL/6 and TLR4$^{-/-}$ mice, evaluating cellular response, humoral response, and lung function. In the bronchoalveolar lavage fluid and lung digests, airway exposure to glyphosate (1 or 100 µg) significantly increased the total cell count, eosinophils, neutrophils, and IgG1 and IgG2a levels. Airway exposure to glyphosate (100 ng, 1 µg, or 100 µg per day for 7 days) also produced substantial pulmonary inflammation, confirmed by histological examination. In addition, glyphosate-rich farm-air samples significantly increased circulating levels of IL-5, IL-10, IL-13 and IL-4 in wildtype and in TLR4$^{-/-}$ mice. Glyphosate was also tested in wildtype mice and significantly increased levels of IL-5, IL-10, IL-13, and IFN-γ (but not IL-4). The glyphosate-induced pro-inflammatory effects were similar to those induced by ovalbumin, and there were no additional or synergistic effects when ovalbumin was co-administered with glyphosate.

Pathological effects of glyphosate on the immune system have been reported in 13-week rat and mouse feeding studies by the NTP (Chan & Mahler, 1992). Relative thymus weight was decreased in male rats exposed for 13 weeks, but increased in male mice. Treatment-related changes in haematological parameters were observed in male rats at 13 weeks and included mild increases in haematocrit [erythrocyte volume fraction] and erythrocytes at 12 500, 25 000, and 50 000 ppm, haemoglobin at 25 000 and 50 000 ppm, and platelets at 50 000 ppm. In female rats, small but significant increases occurred in lymphocyte and platelet counts, leukocytes, mean corpuscular haemoglobin, and mean corpuscular volume at 13 weeks.

Blakley (1997) studied the humoral immune response in female CD-1 mice given drinking-water containing a glyphosate-based formulation at concentrations up to 1.05% for 26 days. The mice were inoculated with sheep erythrocytes to produce a T-lymphocyte, macrophage-dependent antibody response on day 21 of exposure. Antibody production was not affected by the formulation.

(iii) Non-mammalian experimental systems

A positive association between exposure to glyphosate and immunotoxicity in fish has been reported. Kreutz *et al.* (2011) reported alterations

in haematological and immune-system parameters in silver catfish (*Rhamdia quelen*) exposed to sublethal concentrations (10% of the median lethal dose, LC_{50}, at 96 hours) of a glyphosate-based herbicide. Numbers of blood erythrocytes, thrombocytes, lymphocytes, and total leukocytes were significantly reduced after 96 hours of exposure, while the number of immature circulating cells was increased. The phagocytic index, serum bacteria agglutination, and total peroxidase activity were significantly reduced after 24 hours of exposure. Significant decreases in serum bacteria agglutination and lysozyme activity were found after 10 days of exposure. No effect on serum bactericidal and complement natural haemolytic activity was seen after 24 hours or 10 days of exposure to glyphosate.

el-Gendy *et al.* (1998) demonstrated effects of a glyphosate-based formulation (glyphosate, 48%) at 1/1000 of the concentration recommended for field application on humoral and cellular immune response in bolti fish (*Tilapia nilotica*). The mitogenic responses of splenocytes to phytohaemagglutinin, concanavalin A, and lipopolysaccharide in fish exposed to glyphosate for 96 hours were gradually decreased and reached maximum depression after 4 weeks. Glyphosate also produced a concentration-dependent suppression of in-vitro plaque-forming cells in response to sheep erythrocytes.

4.2.4 Cell proliferation and death

(a) Humans

(i) Studies in exposed humans

No data were available to the Working Group.

(ii) Human cells in vitro

Cell proliferation potential was explored in HaCaT keratinocytes exposed to a glyphosate-based formulation (glyphosate, 41%; concentration, up to 0.1 mM) (George & Shukla, 2013). The formulation increased the number of viable cells, as assessed by the MTT assay (based on reduction of the dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) at concentrations up to 0.1 mM, while concentration- and incubation-time-dependent reductions were seen at higher concentrations (up to 1 mM). The formulation (0.01 or 0.1 mM for 72 hours) significantly enhanced cell proliferation (measured by staining for either proliferating cell nuclear antigen or 5-bromo-2′-deoxyuridine); at 0.1 mM, the increases exceeded levels for the positive control, tetradecanoyl-phorbol-13-acetate. The proportion of S-phase cells (assessed using flow cytometry) and the expression of G1/S cell-cycle regulatory proteins (cyclins D1 and E, CDK2, CDK4, and CDK6) increased after exposure to the formulation or the positive control.

Li *et al.* (2013) reported that glyphosate and AMPA inhibited cell growth in eight human cancer cell lines, but not in two immortalized normal prostate cell lines. An ovarian (OVCAR-3) and a prostate (C4–2B) cell line showed the greatest loss in viability, with glyphosate or AMPA at 15–50 mM. Further assays were conducted on AMPA, but not glyphosate, in two prostate cancer cell lines (C4–2B and PC-3), and found cell-cycle arrest (decreased entry of cells into S-phase) and increased apoptosis. [The Working Group noted that the findings from these assays with AMPA are of unclear relevance to the effects of glyphosate.]

Glyphosate (10^{-6} to 1 μM) increased growth by 15–30% relative to controls in hormone-dependent T47D breast cancer cells, but only when endogenous estrogen was minimized in the culture medium (by substitution with 10% dextran-charcoal treated fetal bovine serum). Glyphosate did not affect the growth of hormone-independent MDA-MB231 breast cancer cells cultured in either medium (Thongprakaisang *et al.*, 2013).

Glyphosate (up to 30 μM) did not show cell proliferation potential (5-bromo-2′-deoxyuridine) and did not activate caspase 3 or TP53 in human neuroprogenitor ReN CX cells (Culbreth *et al.*, 2012).

Several studies evaluated the impact of glyphosate or glyphosate-based formulations on apoptotic cell death in the HepG2 human hepatoma cell line. Glyphosate-based formulations induced apoptosis in HepG2 cells, while glyphosate alone was generally without effect or showed effects at considerably higher concentrations (Gasnier *et al.*, 2009, 2010; Mesnage *et al.*, 2013; Chaufan *et al.*, 2014; Coalova *et al.*, 2014). For example, 23.5% of the nuclei of HepG2 cells exposed to a glyphosate-based formulation showed condensed and fragmented chromatin ($P < 0.01$), and caspases 3 and 7 were significantly activated, both effects being indicative of apoptosis (Chaufan *et al.*, 2014). Caspases were unaffected by glyphosate or AMPA alone. Glyphosate and AMPA did not affect cell viability at concentrations up to 1000 mg/L, a concentration that increased rather than decreased cell viability after 48 and 72 hours of incubation. In contrast, cells exposed to glyphosate-based formulation at lower concentrations were not viable. Similarly, Coalova *et al.* (2014) reported that a glyphosate-based formulation (glyphosate, 48%) induced apoptotic cell death in HepG2 cells. Apoptosis was indicated by activation of caspases 3 and 7, and the significant fraction (17.7%) of nuclei with condensed and fragmented chromatin ($P < 0.001$).

In studies with glyphosate and nine different glyphosate-based formulations in three cell lines, glyphosate alone did not increase the activity of adenylate kinase (Mesnage *et al.*, 2013). The activity of caspases 3 and 7 was significantly increased by glyphosate in HepG2 and embryonic kidney HEK293 cells, and elevated (although not significantly) about 1.8 times above control levels in placental choriocarcinoma JEG-3 cells. Two formulations containing an ethoxylated adjuvant induced adenylate kinase activity to a greater extent than caspase activity. All formulations were reported to be more cytotoxic than glyphosate. [In concentration–response curves, glyphosate showed an effect on mitochondrial succinate dehydrogenase activity, a measure of cell viability, that was similar to that shown by one formulation. The calculated 50% lethal concentration in JEG3 cells for mitochondrial succinate dehydrogenase activity was greater for three formulations, although the values appeared inconsistent with the concentration–response curves.]

In HUVEC primary neonate umbilical cord vein cells, and 293 embryonic kidney and JEG3 placental cell lines, Benachour & Séralini (2009) found that glyphosate at relatively high concentrations induced apoptosis, as indicated by induction of caspases 3 and 7, and DNA staining and microscopy. At comparable or lower concentrations, four glyphosate-based formulations all caused primarily necrotic cell death. The umbilical cord HUVEC cells were the most sensitive (by about 100-fold) to the apoptotic effects of glyphosate.

Heu *et al.* (2012) evaluated apoptosis in immortalized human keratinocytes (HaCaT) exposed to glyphosate (5–70 mM). Based on annexin V, propidium iodide and mitochondrial staining, exposures leading to 15% cytotoxicity gave evidence of early apoptosis, while increases in late apoptosis and necrosis were observed at higher levels of cytotoxicity.

(b) Non-human mammalian experimental systems

(i) In vivo

In male Wistar rats, glyphosate (10 mg/kg bw, injected intraperitoneally three times per week for 5 weeks) reduced, but not significantly, the inner mitochondrial membrane integrity of the substantia nigra and cerebral cortex (Astiz *et al.* 2009a). Caspase 3 activity was unaltered in these tissues. Mitochondrial cardiolipin content was significantly reduced, particularly in the substantia nigra, where calpain activity was substantially higher. Glyphosate induced DNA fragmentation in the brain and liver.

(ii) In vitro

In adult Sprague Dawley rat testicular cells exposed in vitro, glyphosate (up to 1%; for 24 or 48 hours) did not provoke cell-membrane alterations (Clair *et al.*, 2012). However, caspase 3 and 7 activity increased with exposure in Sertoli cells alone, and in Sertoli and germ cell mixtures. On the other hand, a glyphosate-based formulation (a 0.1% solution, containing 0.36 g/L of glyphosate) induced membrane alterations and decreased the activity of caspase 3 and 7 in Leydig cells, and in Sertoli and germ cell mixtures. In a separate study, glyphosate increased apoptosis in primary Sertoli cell cultures from mice (Zhao *et al.*, 2013).

Glyphosate (5–40 mM, for 12, 24, 48, or 72 hours) significantly increased cell death in a time- and concentration-dependent manner in differentiated rat pheochromocytoma PC12 (neuronal) cells Gui *et al.* (2012). Apoptotic changes included cell shrinkage, DNA fragmentation, decreased Bcl2 expression, and increased Bax expression. Both autophagy and apoptosis were implicated, as pre-treatment with the pan-caspase inhibitor Z-VAD or the autophagy inhibitor 3-MA inhibited cell loss.

Induction of apoptosis by glyphosate or glyphosate-based formulations was also studied in other cell lines. Glyphosate (10 μM) induced apoptosis in rat heart H9c2 cells, the effect being enhanced when glyphosate was given in combination with the adjuvant TN-20 (5 μM), (Kim *et al.*, 2013). A glyphosate-based formulation induced apoptosis in mouse 3T3-L1 fibroblasts, and inhibited their transformation to adipocytes (Martini *et al.*, 2012). A glyphosate-based formulation (10 mM) did not increase rat hepatoma HTC cell death, but did affect mitochondrial membrane potential (Malatesta *et al.*, 2008).

Glyphosate (up to 30 μM) did not activate caspase 3 or show cell proliferation potential (5-bromo-2'-deoxyuridine) in a mouse neuroprogenitor cell line, but did activate Tp53 at the highest concentration tested (Culbreth *et al.*, 2012).

4.2.5 Other mechanisms

No data on immortalization, epigenetic alterations, altered DNA repair, or genomic instability after exposure to glyphosate were available to the Working Group.

4.3 Data relevant to comparisons across agents and end-points

No data on high-throughput screening or other relevant data were available to the Working Group. Glyphosate was not tested by the Tox21 and ToxCast research programmes of the government of the USA (Kavlock *et al.* 2012; Tice *et al.*, 2013).

4.4 Cancer susceptibility data

No studies that examined genetic, life-stage, or other susceptibility factors with respect to adverse health outcomes that could be associated with exposure to glyphosate were identified by the Working Group.

4.5 Other adverse effects

4.5.1 Humans

In the USA in the past decade, poison-control centres have reported more than 4000 exposures to glyphosate-containing herbicides, of which several hundred were evaluated in a health-care facility, and fatalities were rare (Rumack, 2015). In a pesticide surveillance study carried out by the National Poisons Information Service of the United Kingdom, glyphosate was among the most common pesticide exposure implicated in severe or fatal poisoning cases between 2004 and 2013 (Perry *et al.*, 2014). Deliberate poisonings with glyphosate resulting in toxicity and fatality

have been reported in many countries, including Australia (Stella & Ryan, 2004), Denmark (Mortensen *et al.*, 2000), India (Mahendrakar *et al.*, 2014), Japan (Motoiyuku *et al.*, 2008), Republic of Korea (Park *et al.*, 2013), New Zealand (Temple & Smith, 1992), Sri Lanka (Roberts *et al.*, 2010), Taiwan, China (Chen *et al.*, 2009), and Thailand (Sribanditmongkol *et al.*, 2012).

Glyphosate demonstrated no potential for photo-irritation or photo-sensitization in 346 volunteers exposed dermally on normal or abraded skin (Hayes & Laws, 1991). On the other hand, Mariager *et al.* (2013) reported severe burns after prolonged accidental dermal exposure to a glyphosate-based formulation.

4.5.2 Experimental systems

Glyphosate was tested in nine regulatory submissions included in the Toxicity Reference Database (ToxRefDB) and reviewed by the EPA (EPA, 2015). Specifically, study design, treatment group, and treatment-related effect information were captured for four long-term studies and/or carcinogenicity studies, one short-term study, two multigeneration studies of reproductivity, and two studies of developmental toxicity. The NTP also tested glyphosate in a 13-week study in rats and mice (Chan & Mahler, 1992).

In a long-term combined study of toxicity and carcinogenicity in rats given glyphosate at nominal doses of 100, 400, and 1000 mg/kg bw per day, inflammation was observed in the stomach mucosa of females at the intermediate and highest doses (EPA, 1990, 1991b). In males at the highest dose, liver weight, cataracts and lens degeneration in the eyes, and urine specific gravity were increased, while body weight, body-weight gain, and urinary pH were decreased. Pancreatic acinar cell atrophy was observed in males at the highest dose. Pancreatic inflammation was also observed in male rats at the highest dose in a short-term study (nominal doses of 50, 250, and 1000 mg/kg bw per day) (EPA, 1987). In the study by the NTP, cytoplasmic alteration was observed in the parotid and submandibular salivary glands of rats (Chan & Mahler, 1992).

In a study of carcinogenicity in mice given glyphosate at doses of 150, 1500, or 4500 mg/kg bw per day, liver hypertrophy and necrosis were observed in males at the highest dose (EPA, 1983). Other effects in males at the highest dose included increased testes weight, interstitial nephritis, and decreased body weight. In females at the highest dose, ovary weights were increased, proximal tubule epithelial basophilia and hypertrophy was observed, and body weights were decreased. In the study by the NTP, cytoplasmic alteration was observed in the parotid salivary glands in mice (Chan & Mahler, 1992).

Developmental and reproductive toxicity

In a study of developmental toxicity in rats given glyphosate at a dose of 300, 1000, or 3500 mg/kg bw per day, reduced implantation rates and fewer live fetuses were observed in dams at the highest dose (EPA, 1980b). In fetuses at the highest dose, unossified sternebra were observed and fetal weight was reduced.

5. Summary of Data Reported

5.1 Exposure data

Glyphosate is a broad-spectrum herbicide that is effective at killing or suppressing all plant types, including grasses, perennials, and woody plants. The herbicidal activity of glyphosate was discovered in 1970 and since then its use has increased to a point where it is now the most heavily used herbicide in the world, with an annual global production volume in 2012 of more than 700 000 tonnes used in more than 750 different products. Changes in farming practice and the development of genetically modified crops that are resistant to glyphosate have contributed to the increase in use.

There is little information available on occupational or community exposure to glyphosate. Glyphosate can be found in soil, air, surface water and groundwater, as well as in food. It has been detected in air during agricultural herbicide-spraying operations. Glyphosate was detected in urine in two studies of farmers in the USA, in urban populations in Europe, and in a rural population living near areas sprayed for drug eradication in Columbia. However, urinary concentrations were mostly below the limit of detection in several earlier studies of forestry workers who sprayed glyphosate. Exposure of the general population occurs mainly through diet.

5.2 Human carcinogenicity data

In its evaluation of the epidemiological studies reporting on cancer risks associated with exposure to glyphosate, the Working Group identified seven reports from the Agricultural Health Study (AHS) cohort and several reports from case–control studies. The AHS cohort, the pooled analyses of the case–control studies in the midwest USA, and the cross-Canada study were considered key investigations because of their relatively large size. Reports from two or more independent studies were available for non-Hodgkin lymphoma (NHL), multiple myeloma, Hodgkin lymphoma, glioma, and prostate. For the other cancer sites, results from only one study were available for evaluation.

5.2.1 NHL and other haematopoietic cancers

Two large case–control studies of NHL from Canada and the USA, and two case–control studies from Sweden reported statistically significant increased risks of NHL in association with exposure to glyphosate. For the study in Canada, the association was seen among those with more than 2 days/year of exposure, but no adjustment for other pesticides was done. The other three studies reported excesses for NHL associated with exposure to glyphosate, after adjustment for other pesticides (reported odds ratio were 2.1 (95% CI, 1.1–4.0); 1.85 (95% CI, 0.55–6.2); and 1.51 (95% CI, 0.77–2.94). Subtype-specific analyses in a Swedish case–control study indicated positive associations for total NHL, as well as all subtypes, but this association was statistically significant only for the subgroup of lymphocytic lymphoma/chronic lymphocytic leukaemia (OR, 3.35; 95% CI, 1.42–7.89). An elevated risk (OR, 3.1; 95% CI, 0.6–17.1) was also found for B-cell lymphoma in an European study based on few cases. One hospital-based case–control study from France did not find an association between exposure to glyphosate and NHL (OR, 1.0; 95% CI, 0.5–2.2) based on few exposed cases.

A roughly twofold excess of multiple myeloma, a subtype of NHL, was reported in three studies: only among the highest category of glyphosate use (> 2 days/year) in the large Canadian case–control study, in a case–control study from Iowa, USA, and in a French case–control study (all not statistically significant). These three studies did not adjust for the effect of other pesticides. In the AHS, there was no association with NHL (OR, 1.1; 0.7–1.9). For multiple myeloma, relative risk was 1.1 (95% CI, 0.5–2.4) when adjusted for age only; but was 2.6 (95% CI, 0.7–9.4) when adjusted for multiple confounders. No excess in leukaemia was observed in a case–control study in Iowa and Minnesota, USA, or in the AHS.

In summary, case–control studies in the USA, Canada, and Sweden reported increased risks for NHL associated with exposure to glyphosate. The increased risk persisted in the studies that adjusted for exposure to other pesticides. The AHS cohort did not show an excess of NHL. The Working Group noted that there were excesses reported for multiple myeloma in three studies; however, they did not weight this evidence as strongly as that of NHL because of the possibility that chance could not be excluded; none of the

risk estimates were statistically significant nor were they adjusted for other pesticide exposures.

5.2.2. Other cancer sites

No association of glyphosate with cancer of the brain in adults was found in the Upper Midwest Health case–control study. No associations in single case–control studies were found for cancers of the oesophagus and stomach, prostate, and soft-tissue sarcoma. For all other cancer sites (lung, oral cavity, colorectal, pancreas, kidney, bladder, breast, prostate, melanoma) investigated in the large AHS, no association with exposure to glyphosate was found.

5.3 Animal carcinogenicity data

Glyphosate was tested for carcinogenicity in male and female mice by dietary administration in two studies, and in male and female rats by dietary administration in five studies and in drinking-water in one study. A glyphosate-based formulation was also tested in drinking-water in one study in male and female rats, and by skin application in one initiation–promotion study in male mice.

There was a positive trend in the incidence of renal tubule carcinoma and of renal tubule adenoma or carcinoma (combined) in males in one feeding study in CD-1 mice. Renal tubule carcinoma is a rare tumour in this strain of mice. No significant increase in tumour incidence was seen in female mice in this study. In the second feeding study, there was a significant positive trend in the incidence of haemangiosarcoma in male CD-1 mice. No significant increase in tumour incidence was seen in female mice in this study.

For the five feeding studies in rats, two studies in the Sprague-Dawley strain showed a significant increase in the incidence of pancreatic islet cell adenoma in males – one of these two studies also showed a significant positive trend in the incidences of hepatocellular adenoma in males and of thyroid C-cell adenoma in females. Two studies (one in Sprague-Dawley rats, one in Wistar rats) found no significant increase in tumour incidence at any site. One study in Wistar rats was inadequate for the evaluation because of the short duration of exposure.

In the study in Wistar rats given drinking-water containing glyphosate, there was no significant increase in tumour incidence.

A glyphosate-based formulation was found to be a skin-tumour promoter in the initiation–promotion study in male Swiss mice. The study of a glyphosate-based formulation in drinking-water in Sprague-Dawley rats was inadequate for the evaluation because of the small number of animals per group, and the limited information provided on tumour histopathology and incidence in individual animals. These studies of a chemical mixture containing glyphosate were considered inadequate to evaluate the carcinogenicity of glyphosate alone.

5.4. Other relevant data

Direct data on absorption of glyphosate in humans were not available to the Working Group. Glyphosate was detected in the urine of agricultural workers in several studies, and in the blood of poisoning cases, indicative of absorption. Some evidence for absorption through human skin (~2%) was reported in studies in vitro. The minor role of dermal absorption was also shown in a study in non-human primate model in vivo. However, no study examined the rates of absorption in humans. In rodents, several studies showed up to 40% absorption after oral administration of a single or repeated dose.

Glyphosate was measured in human blood. No data on parenchymal tissue distribution for glyphosate in humans were available to the Working Group. In rats given glyphosate by oral administration, concentrations in tissues had the following rank order: kidneys > spleen > fat > liver. Repeated administration had no effect

on the distribution of glyphosate. In a study in rats, the half-life of glyphosate in plasma was estimated to be more than 1 day, indicating that glyphosate is not rapidly eliminated.

In the environment, glyphosate is degraded by soil microbes, primarily to aminomethylphosphonic acid (AMPA) and carbon dioxide. Glyphosate is not efficiently metabolized in humans or other mammals. In rats, small amounts of AMPA were detected in the plasma and in the colon, with the latter being attributed to intestinal microbial metabolism. In humans, small amounts of AMPA are detectable in blood in cases of deliberate glyphosate poisoning. Few studies examined the possible effects of glyphosate-based formulations on metabolizing enzymes, but no firm conclusions could be drawn from these studies.

Studies in rodents showed that systemically absorbed glyphosate is excreted unchanged into the urine, and that the greatest amount is excreted in the faeces, indicating poor absorption. Glyphosate was detected in the urine of humans who were exposed occupationally to glyphosate. AMPA has also been detected in human urine.

Glyphosate is not electrophilic.

A large number of studies examined a wide range of end-points relevant to genotoxicity with glyphosate alone, glyphosate-based formulations, and AMPA.

There is strong evidence that glyphosate causes genotoxicity. The evidence base includes studies that gave largely positive results in human cells in vitro, in mammalian model systems in vivo and in vitro, and studies in other non-mammalian organisms. In-vivo studies in mammals gave generally positive results in the liver, with mixed results for the kidney and bone marrow. The end-points that have been evaluated in these studies comprise biomarkers of DNA adducts and various types of chromosomal damage. Tests in bacterial assays gave consistently negative results.

The evidence for genotoxicity caused by glyphosate-based formulations is strong. There were three studies of genotoxicity end-points in community residents exposed to glyphosate-based formulations, two of which reported positive associations. One of these studies examined chromosomal damage (micronucleus formation) in circulating blood cells before and after aerial spraying with glyphosate-based formulations and found a significant increase in micronucleus formation after exposure in three out of four different geographical areas. Additional evidence came from studies that gave largely positive results in human cells in vitro, in mammalian model systems in vivo and in vitro, and studies in other non-mammalian organisms. The end-points that were evaluated in these studies comprised biomarkers of DNA adducts and various types of chromosomal damage. The pattern of tissue specificity of genotoxicity end-points observed with glyphosate-based formulations is similar to that observed with glyphosate alone. Tests in bacterial assays gave generally negative results.

For AMPA, the evidence for genotoxicity is moderate. While the number of studies that examined the effects of AMPA was not large, all of the studies gave positive results. Specifically, genotoxicity was reported in a study in humans in vitro, a study in mammals in vivo, a study in mammals in vitro, and one study in eels in vivo.

Strong evidence exists that glyphosate, AMPA, and glyphosate-based formulations can induce oxidative stress. Evidence came from studies in many rodent tissues in vivo, and human cells in vitro. In some of these studies, the mechanism was challenged by co-administration of antioxidants and observed amelioration of the effects. Similar findings have been reported in fish and other aquatic species. Various end-points (e.g. lipid peroxidation markers, oxidative DNA adducts, dysregulation of antioxidant enzymes) have been evaluated in numerous studies. This

increased the confidence of the Working Group in the overall database.

There is weak evidence that glyphosate or glyphosate-based formulations induce receptor-mediated effects. In multiple experiments, glyphosate-based formulations affected aromatase activity; glyphosate was active in a few of these studies. Some activity in other nuclear receptor-mediated pathways has been observed for glyphosate or glyphosate-based formulations. In one series of experiments, glyphosate was not found to be a ligand to several receptors and related proteins (aryl hydrocarbon receptor, peroxisome proliferator-activated receptors, pregnane X receptor).

There is weak evidence that glyphosate may affect cell proliferation or death. Several studies in human and rodent cell lines have reported cytotoxicity and cell death, the latter attributed to the apoptosis pathway. Studies that examined the effects of glyphosate alone or a glyphosate-based formulation found that glyphosate alone had no effect, or a weaker effect than the formulation.

There is weak evidence that glyphosate may affect the immune system, both the humoral and cellular response, upon long-term treatment in rodents. Several studies in fish, with glyphosate or its formulations, also reported immunosuppressive effects.

With regard to the other key characteristics of human carcinogens (IARC, 2014), the Working Group considered that the data were too few for an evaluation to be made.

Severe or fatal human poisoning cases have been documented worldwide. In rodents, organ and systemic toxicity from exposures to glyphosate are demonstrated by liver-weight effects and necrosis in animals at high doses. Additionally, effects on the pancreas, testes, kidney and ovaries, as well as reduced implantations and unossified sternebra were seen at similar doses.

No data on cancer-related susceptibility after exposure to glyphosate were available to the Working Group.

Overall, the mechanistic data provide strong evidence for genotoxicity and oxidative stress. There is evidence that these effects can operate in humans.

6. Evaluation

6.1 Cancer in humans

There is *limited evidence* in humans for the carcinogenicity of glyphosate. A positive association has been observed for non-Hodgkin lymphoma.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of glyphosate.

6.3 Overall evaluation

Glyphosate is *probably carcinogenic to humans (Group 2A).*

6.4 Rationale

In making this overall evaluation, the Working Group noted that the mechanistic and other relevant data support the classification of glyphosate in Group 2A.

In addition to limited evidence for the carcinogenicity of glyphosate in humans and sufficient evidence for the carcinogenicity of glyphosate in experimental animals, there is strong evidence that glyphosate can operate through two key characteristics of known human carcinogens, and that these can be operative in humans. Specifically:

- There is strong evidence that exposure to glyphosate or glyphosate-based formulations is genotoxic based on studies in humans in vitro and studies in experimental animals.

One study in several communities in individuals exposed to glyphosate-based formulations also found chromosomal damage in blood cells; in this study, markers of chromosomal damage (micronucleus formation) were significantly greater after exposure than before exposure in the same individuals.

- There is strong evidence that glyphosate, glyphosate-based formulations, and aminomethylphosphonic acid can act to induce oxidative stress based on studies in experimental animals, and in studies in humans in vitro. This mechanism has been challenged experimentally by administering antioxidants, which abrogated the effects of glyphosate on oxidative stress. Studies in aquatic species provide additional evidence for glyphosate-induced oxidative stress.

References

Abraxis (2005). Glyphosate Plate Kit Part No. 500086. Warminster (PA): Abraxis, LLC. Available from: http://www.abraxiskits.com/uploads/products/docfiles/184PN500086USER.pdf, accessed 28 July 2015.

Acquavella JF, Alexander BH, Mandel JS, Gustin C, Baker B, Chapman P *et al.* (2004). Glyphosate biomonitoring for farmers and their families: results from the Farm Family Exposure Study. *Environ Health Perspect*, 112(3):321–6. doi:10.1289/ehp.6667 PMID:14998747

Akcha F, Spagnol C, Rouxel J (2012). Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos. *Aquat Toxicol*, 106–107:104–13. doi:10.1016/j.aquatox.2011.10.018 PMID:22115909

Alavanja MC, Samanic C, Dosemeci M, Lubin J, Tarone R, Lynch CF *et al.* (2003). Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort. *Am J Epidemiol*, 157(9):800–14. doi:10.1093/aje/kwg040 PMID:12727674

Alavanja MC, Sandler DP, McMaster SB, Zahm SH, McDonnell CJ, Lynch CF *et al.* (1996). The Agricultural Health Study. *Environ Health Perspect*, 104(4):362–9. doi:10.1289/ehp.96104362 PMID:8732939

Alvarez-Moya C, Silva MR, Arámbula AR, Sandoval AI, Vasquez HC, González Montes RM (2011). Evaluation of genetic damage induced by glyphosate isopropylamine salt using *Tradescantia* bioassays. *Genet Mol Biol*, 34(1):127–30. doi:10.1590/S1415-47572010005000108 PMID:21637555

Alvarez-Moya C, Silva MR, Ramírez CV, Gallardo DG, Sánchez RL, Aguirre AC *et al.* (2014). Comparison of the *in vivo* and *in vitro* genotoxicity of glyphosate isopropylamine salt in three different organisms. *Genet Mol Biol*, 37(1):105–10. doi:10.1590/S1415-47572014000100016 PMID:24688297

Andreotti G, Freeman LE, Hou L, Coble J, Rusiecki J, Hoppin JA *et al.* (2009). Agricultural pesticide use and pancreatic cancer risk in the Agricultural Health Study Cohort. *Int J Cancer*, 124(10):2495–500. doi:10.1002/ijc.24185 PMID:19142867

Aris A, Leblanc S (2011). Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. *Reprod Toxicol*, 31(4):528–33. doi:10.1016/j.reprotox.2011.02.004 PMID:21338670

Astiz M, de Alaniz MJ, Marra CA (2009a). Effect of pesticides on cell survival in liver and brain rat tissues. *Ecotoxicol Environ Saf*, 72(7):2025–32. doi:10.1016/j.ecoenv.2009.05.001 PMID:19493570

Astiz M, de Alaniz MJ, Marra CA (2009b). Antioxidant defense system in rats simultaneously intoxicated with agrochemicals. *Environ Toxicol Pharmacol*, 28(3):465–73. doi:10.1016/j.etap.2009.07.009 PMID:21784044

Astiz M, Hurtado de Catalfo GE, García MN, Galletti SM, Errecalde AL, de Alaniz MJ *et al.* (2013). Pesticide-induced decrease in rat testicular steroidogenesis is differentially prevented by lipoate and tocopherol. *Ecotoxicol Environ Saf*, 91:129–38. doi:10.1016/j.ecoenv.2013.01.022 PMID:23465731

Band PR, Abanto Z, Bert J, Lang B, Fang R, Gallagher RP *et al.* (2011). Prostate cancer risk and exposure to pesticides in British Columbia farmers. *Prostate*, 71(2):168–83. doi:10.1002/pros.21232 PMID:20799287

Battaglin WA, Kolpin DW, Scribner EA, Kuivila KM, Sandstrom MW (2005). Glyphosate, other herbicides, and transformation products in midwestern streams, 20021. *J Am Water Resour Assoc*, 41(2):323–32. doi:10.1111/j.1752-1688.2005.tb03738.x

Benachour N, Séralini GE (2009). Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. *Chem Res Toxicol*, 22(1):97–105. doi:10.1021/tx800218n PMID:19105591

Benachour N, Sipahutar H, Moslemi S, Gasnier C, Travert C, Séralini GE (2007). Time- and dose-dependent effects of Roundup on human embryonic and placental cells. *Arch Environ Contam Toxicol*, 53(1):126–33. doi:10.1007/s00244-006-0154-8 PMID:17486286

Bernal J, Bernal JL, Martin MT, Nozal MJ, Anadón A, Martínez-Larrañaga MR *et al.* (2010). Development and validation of a liquid chromatography-fluorescence-mass spectrometry method to measure glyphosate and aminomethylphosphonic acid in rat plasma. *J Chromatogr B Analyt Technol Biomed Life*

Sci, 878(31):3290–6. doi:10.1016/j.jchromb.2010.10.013 PMID:21106459

Blair A, Thomas K, Coble J, Sandler DP, Hines CJ, Lynch CF *et al.* (2011). Impact of pesticide exposure misclassification on estimates of relative risks in the Agricultural Health Study. *Occup Environ Med*, 68(7):537–41. doi:10.1136/oem.2010.059469 PMID:21257983

Blakley BR (1997). Effect of Roundup and Tordon 202C herbicides on antibody production in mice. *Vet Hum Toxicol*, 39(4):204–6. PMID:9251167

Bolognesi C, Bonatti S, Degan P, Gallerani E, Peluso M, Rabboni R *et al.* (1997). Genotoxic activity of glyphosate and its technical formulation Roundup. *J Agric Food Chem*, 45(5):1957–62. doi:10.1021/jf9606518

Bolognesi C, Carrasquilla G, Volpi S, Solomon KR, Marshall EJ (2009). Biomonitoring of genotoxic risk in agricultural workers from five Colombian regions: association to occupational exposure to glyphosate. *J Toxicol Environ Health A*, 72(15–16):986–97. doi:10.1080/15287390902929741 PMID:19672767

Bonini MG, Rota C, Tomasi A, Mason RP (2006). The oxidation of 2′,7′-dichlorofluorescein to reactive oxygen species: a self-fulfilling prophesy? *Free Radic Biol Med*, 40(6):968–75. doi:10.1016/j.freeradbiomed.2005.10.042 PMID:16540392

Borggaard OK, Gimsing AL (2008). Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. *Pest Manag Sci*, 64(4):441–56. doi:10.1002/ps.1512 PMID:18161065

Botero-Coy AM, Ibáñez M, Sancho JV, Hernández F (2013). Improvements in the analytical methodology for the residue determination of the herbicide glyphosate in soils by liquid chromatography coupled to mass spectrometry. *J Chromatogr A*, 1292:132–41. doi:10.1016/j.chroma.2012.12.007 PMID:23332301

Botero-Coy AM, Ibáñez M, Sancho JV, Hernández F (2013b). Direct liquid chromatography-tandem mass spectrometry determination of underivatized glyphosate in rice, maize and soybean. *J Chromatogr A*, 1313:157–65. doi:10.1016/j.chroma.2013.07.037 PMID:23891211

Brewster DW, Warren J, Hopkins WE 2nd (1991). Metabolism of glyphosate in Sprague-Dawley rats: tissue distribution, identification, and quantitation of glyphosate-derived materials following a single oral dose. *Fundam Appl Toxicol*, 17(1):43–51. doi:10.1016/0272-0590(91)90237-X PMID:1916078

Brown LM, Blair A, Gibson R, Everett GD, Cantor KP, Schuman LM *et al.* (1990). Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. *Cancer Res*, 50(20):6585–91. PMID:2208120

Brown LM, Burmeister LF, Everett GD, Blair A (1993). Pesticide exposures and multiple myeloma in Iowa men. *Cancer Causes Control*, 4(2):153–6. doi:10.1007/BF00053156 PMID:8481493

Brüch W, Rosenborg AE, Johler RK, Gudmunsson L, Nielsen CB, Plauborg F, et al. (2013). Monitoring results 1999–2012. The Danish Pesticide Leaching Assessment Programme. Available from: http://pesticidvarsling.dk/publ_result/index.html, accessed 1 December 2014.

Cantor KP, Blair A, Everett G, Gibson R, Burmeister LF, Brown LM *et al.* (1992). Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. *Cancer Res*, 52(9):2447–55. PMID:1568215

Carreón T, Butler MA, Ruder AM, Waters MA, Davis-King KE, Calvert GM *et al.*; Brain Cancer Collaborative Study Group(2005). Gliomas and farm pesticide exposure in women: the Upper Midwest Health Study. *Environ Health Perspect*, 113(5):546–51. doi:10.1289/ehp.7456 PMID:15866761

Cattaneo R, Clasen B, Loro VL, de Menezes CC, Pretto A, Baldisserotto B *et al.* (2011). Toxicological responses of *Cyprinus carpio* exposed to a commercial formulation containing glyphosate. *Bull Environ Contam Toxicol*, 87(6):597–602. doi:10.1007/s00128-011-0396-7 PMID:21931962

Cattani D, de Liz Oliveira Cavalli VL, Heinz Rieg CE, Domingues JT, Dal-Cim T, Tasca CI *et al.* (2014). Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: involvement of glutamate excitotoxicity. *Toxicology*, 320:34–45. doi:10.1016/j.tox.2014.03.001 PMID:24636977

Cavalcante DG, Martinez CB, Sofia SH (2008). Genotoxic effects of Roundup on the fish *Prochiloduslineatus*. *Mutat Res*, 655(1–2):41–6. doi:10.1016/j.mrgentox.2008.06.010 PMID:18638566

Cavaş T, Könen S (2007). Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (*Carassius auratus*) exposed to a glyphosate formulation using the micronucleus test and the comet assay. *Mutagenesis*, 22(4):263–8. doi:10.1093/mutage/gem012 PMID:17426049

Cavuşoğlu K, Yapar K, Oruç E, Yalçın E (2011). Protective effect of *Ginkgo biloba* L. leaf extract against glyphosate toxicity in Swiss albino mice. *J Med Food*, 14(10):1263–72. doi:10.1089/jmf.2010.0202 PMID:21859351

CCM International (2011). Outlook for China glyphosate industry 2012–2016. Available from: http://www.researchandmarkets.com/reports/2101356/outlook_for_china_glyphosate_industry_20122016, accessed 28 July 2015.

Centre de Toxicologie du Québec (1988). Etude de l'exposition professionelle des travailleurs forestiers exposés au glyphosate. Quebec: Le Centre Hospitalier de l'Université Laval. Available from: http://www.santecom.qc.ca/Bibliothequevirtuelle/santecom/35567000039898.pdf, accessed 28 July 2015. [French]

Chan P, Mahler J (1992). NTP technical report on the toxicity studies of glyphosate (CAS No. 1071–83–6)

administered in dosed feed to F344/N rats and B6C3F1 mice. *Toxic Rep Ser*, 16:1–58. PMID:12209170

Chandra M, Frith CH (1994). Spontaneous renal lesions in CD-1 and B6C3F1 mice. *Exp Toxicol Pathol*, 46(3):189–98. doi:10.1016/S0940-2993(11)80080-1 PMID:8000238

Chang FC, Simcik MF, Capel PD (2011). Occurrence and fate of the herbicide glyphosate and its degradate aminomethylphosphonic acid in the atmosphere. *Environ Toxicol Chem*, 30(3):548–55. doi:10.1002/etc.431 PMID:21128261

Chaufan G, Coalova I, Ríos de Molina MC (2014). Glyphosate commercial formulation causes cytotoxicity, oxidative effects, and apoptosis on human cells: differences with its active ingredient. *Int J Toxicol*, 33(1):29–38. doi:10.1177/1091581813517906 PMID:24434723

Chen L, Xie M, Bi Y, Wang G, Deng S, Liu Y (2012). The combined effects of UV-B radiation and herbicides on photosynthesis, antioxidant enzymes and DNA damage in two bloom-forming cyanobacteria. *Ecotoxicol Environ Saf*, 80:224–30. doi:10.1016/j.ecoenv.2012.03.007 PMID:22464588

Chen M-X, Cao Z-Y, Jiang Y, Zhu Z-W (2013). Direct determination of glyphosate and its major metabolite, aminomethylphosphonic acid, in fruits and vegetables by mixed-mode hydrophilic interaction/weak anion-exchange liquid chromatography coupled with electrospray tandem mass spectrometry. *J Chromatogr A*, 1272:90–9. doi:10.1016/j.chroma.2012.11.069 PMID:23261284

Chen YJ, Wu ML, Deng JF, Yang CC (2009). The epidemiology of glyphosate-surfactant herbicide poisoning in Taiwan, 1986–2007: a poison center study. *Clin Toxicol (Phila)*, 47(7):670–7. doi:10.1080/15563650903140399 PMID:19640238

Chruscielska K, Brzezinski J, Kita K, Kalhorn D, Kita I, Graffstein B *et al.* (2000). Glyphosate - Evaluation of chronic activity and possible far-reaching effects. Part 1. Studies on chronic toxicity. *Pestycydy (Warsaw)*, 3–4:11–20.

Clair E, Mesnage R, Travert C, Séralini GÉ (2012). A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells in vitro, and testosterone decrease at lower levels. *Toxicol In Vitro*, 26(2):269–79. doi:10.1016/j.tiv.2011.12.009 PMID:22200534

Clements C, Ralph S, Petras M (1997). Genotoxicity of select herbicides in *Rana catesbeiana* tadpoles using the alkaline single-cell gel DNA electrophoresis (comet) assay. *Environ Mol Mutagen*, 29(3):277–88. doi:10.1002/(SICI)1098-2280(1997)29:3<277::AID-EM8>3.0.CO;2-9 PMID:9142171

Coalova I, Ríos de Molina MC, Chaufan G (2014). Influence of the spray adjuvant on the toxicity effects of a glyphosate formulation. *Toxicol In Vitro*, 28(7):1306–11. doi:10.1016/j.tiv.2014.06.014 PMID:24999230

Cocco P, Satta G, Dubois S, Pili C, Pilleri M, Zucca M *et al.* (2013). Lymphoma risk and occupational exposure to pesticides: results of the Epilymph study. *Occup Environ Med*, 70(2):91–8. doi:10.1136/oemed-2012-100845 PMID:23117219

ColomboPage News Desk (2014). Sri Lanka lifts ban on sale of glyphosate. ColomboPage, Sri Lanka Internet Newspaper [online newspaper]. 13 May, 12:13 am Sri Lanka time. Available from: http://www.colombopage.com/archive_14A/May13_1399920230CH.php, accessed June 2015.

Conners DE, Black MC (2004). Evaluation of lethality and genotoxicity in the freshwater mussel *Utterbackia imbecillis* (Bivalvia: Unionidae) exposed singly and in combination to chemicals used in lawn care. *Arch Environ Contam Toxicol*, 46(3):362–71. doi:10.1007/s00244-003-3003-z PMID:15195808

Costa MJ, Monteiro DA, Oliveira-Neto AL, Rantin FT, Kalinin AL (2008). Oxidative stress biomarkers and heart function in bullfrog tadpoles exposed to Roundup Original. *Ecotoxicology*, 17(3):153–63. doi:10.1007/s10646-007-0178-5 PMID:17987383

Culbreth ME, Harrill JA, Freudenrich TM, Mundy WR, Shafer TJ (2012). Comparison of chemical-induced changes in proliferation and apoptosis in human and mouse neuroprogenitor cells. *Neurotoxicology*, 33(6):1499–510. doi:10.1016/j.neuro.2012.05.012 PMID:22634143

Curwin BD, Hein MJ, Sanderson WT, Nishioka MG, Reynolds SJ, Ward EM *et al.* (2005). Pesticide contamination inside farm and nonfarm homes. *J Occup Environ Hyg*, 2(7):357–67. doi:10.1080/15459620591001606 PMID:16020099

Curwin BD, Hein MJ, Sanderson WT, Striley C, Heederik D, Kromhout H *et al.* (2007). Urinary pesticide concentrations among children, mothers and fathers living in farm and non-farm households in Iowa. *Ann Occup Hyg*, 51(1):53–65. doi:10.1093/annhyg/mel062 PMID:16984946

de Castilhos Ghisi N, Cestari MM (2013). Genotoxic effects of the herbicide Roundup(®) in the fish *Corydoras paleatus* (Jenyns 1842) after short-term, environmentally low concentration exposure. *Environ Monit Assess*, 185(4):3201–7. doi:10.1007/s10661-012-2783-x PMID:22821326

De Marco A, De Simone C, Raglione M, Testa A, Trinca S (1992). Importance of the type of soil for the induction of micronuclei and the growth of primary roots of *Vicia faba* treated with the herbicides atrazine, glyphosate and maleic hydrazide. *Mutat Res*, 279(1):9–13. doi:10.1016/0165-1218(92)90260-7 PMID:1374535

de Menezes CC, da Fonseca MB, Loro VL, Santi A, Cattaneo R, Clasen B *et al.* (2011). Roundup effects on oxidative stress parameters and recovery pattern of *Rhamdia quelen*. *Arch Environ Contam Toxicol*, 60(4):665–71. doi:10.1007/s00244-010-9574-6 PMID:20680259

De Roos AJ, Blair A, Rusiecki JA, Hoppin JA, Svec M, Dosemeci M *et al.* (2005a). Cancer incidence among glyphosate-exposed pesticide applicators in the Agricultural Health Study. *Environ Health Perspect*, 113(1):49–54. doi:10.1289/ehp.7340 PMID:15626647

De Roos AJ, Svec MA, Blair A, Rusiecki JA, Dosemeci M, Alavanja MC *et al.* (2005b). Glyphosate results revisited: De Roos et al. respond. *Environ Health Perspect*, 113(6):A366–7. doi:10.1289/ehp.113-a366

De Roos AJ, Zahm SH, Cantor KP, Weisenburger DD, Holmes FF, Burmeister LF *et al.* (2003). Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. *Occup Environ Med*, 60(9):E11 doi:10.1136/oem.60.9.e11 PMID:12937207

De Souza Filho J, Sousa CC, Da Silva CC, De Sabóia-Morais SM, Grisolia CK (2013). Mutagenicity and genotoxicity in gill erythrocyte cells of *Poecilia reticulata* exposed to a glyphosate formulation. *Bull Environ Contam Toxicol*, 91(5):583–7. doi:10.1007/s00128-013-1103-7 PMID:24042842

Dennis LK, Lynch CF, Sandler DP, Alavanja MC (2010). Pesticide use and cutaneous melanoma in pesticide applicators in the Agricultural Health Study. *Environ Health Perspect*, 118(6):812–7. doi:10.1289/ehp.0901518 PMID:20164001

Dill GM, Sammons RD, Feng PCC, Kohn F, Kretzmer K, Mehrsheikh A *et al.* (2010). Chapter 1: Glyphosate: discovery, development, applications, and properties. In: Nandula VK editor. *Glyphosate resistance in crops and weeds: history, development, and management*. Hoboken (NJ): Wiley; pp. 1–33.

Dimitrov BD, Gadeva PG, Benova DK, Bineva MV (2006). Comparative genotoxicity of the herbicides Roundup, Stomp and Reglone in plant and mammalian test systems. *Mutagenesis*, 21(6):375–82. doi:10.1093/mutage/gel044 PMID:16998229

dos Santos KC, Martinez CB (2014). Genotoxic and biochemical effects of atrazine and Roundup(®), alone and in combination, on the Asian clam *Corbicula fluminea*. *Ecotoxicol Environ Saf*, 100:7–14. doi:10.1016/j.ecoenv.2013.11.014 PMID:24433785

Duke SO, Powles SB (2009). Glyphosate-resistant crops and weeds. Now and in the future. *AgBioForum*, 12(3&4):346–57.

EFSA (2009). 2007 Annual Report on Pesticide Residues according to Article 32 of Regulation (EC) No 396/2005. Parma: European Food Safety Authority. Available from: http://www.efsa.europa.eu/en/efsajournal/pub/305r.htm, accessed 1 November 2014.

el-Gendy KS, Aly NM, el-Sebae AH (1998). Effects of edifenphos and glyphosate on the immune response and protein biosynthesis of bolti fish (*Tilapia nilotica*). *J Environ Sci Health B*, 33(2):135–49. doi:10.1080/03601239809373135 PMID:9536512

Elie-Caille C, Heu C, Guyon C, Nicod L (2010). Morphological damages of a glyphosate-treated human keratinocyte cell line revealed by a micro- to nanoscale microscopic investigation. *Cell Biol Toxicol*, 26(4):331–9. doi:10.1007/s10565-009-9146-6 PMID:20043237

Engel LS, Hill DA, Hoppin JA, Lubin JH, Lynch CF, Pierce J *et al.* (2005). Pesticide use and breast cancer risk among farmers' wives in the Agricultural Health Study. *Am J Epidemiol*, 161(2):121–35. doi:10.1093/aje/kwi022 PMID:15632262

EPA (1980a). Glyphosate; Submission of rat teratology, rabbit teratology, dominant lethal mutagenicity assay in mice. Washington (DC): United States Environmental Protection Agency, Office of Toxic substances. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-090.pdf, accessed 10 March 2015.

EPA (1980b). Review of Rodwell DE, Tasker EJ, Blair AM, *et al.* (1980). Teratology study in rats: IRDC No. 401–054. MRID 00046362. Washington (DC): United States Environmental Protection Agency. Available from: http://www.epa.gov/ncct/toxrefdb/, and from http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-090.pdf, accessed 10 March 2015.

EPA (1983). Review of Knezevich A, Hogan G (1983). A chronic feeding study of glyphosate (Roundup Technical) in mice: Project No. 77–2061: Bdn-77- 420. Final Report. MRID 00130406. Washington (DC): United States Environmental Protection Agency. Available from: http://www.epa.gov/ncct/toxrefdb/, accessed 10 March 2015.

EPA (1985a). Glyphosate; EPA Reg.#: 524–308; Mouse oncogenicity study. Document No. 004370. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-183.pdf, accessed 10 March 2015.

EPA (1985b). EPA Reg.#: 524–308; Roundup; glyphosate; pathology report on additional kidney sections. Document No. 004855. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-206.pdf, accessed 10 March 2015.

EPA (1986). Glyphosate; EPA Registration No. 524–308; Roundup; additional histopathological evaluations of kidneys in the chronic feeding study of glyphosate in mice. Document No. 005590. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/

reviews/103601/103601-211.pdf, accessed 10 March 2015.

EPA (1987). Review of Stout L, Johnson C (1987). 90-Day study of glyphosate administered in feed to Sprague-Dawley rats: Proj. ID ML-86-351/EHL 86128. MRID 40559401. Washington (DC): United States Environmental Protection Agency. Available from: http://www.epa.gov/ncct/toxrefdb/, accessed 10 March 2015.

EPA (1990). Review of Stout L, Ruecker F (1990). Chronic study of glyphosate administered in feed to albino rats: Laboratory Project Number: Msl-10495: RD 1014. MRID 41643801. Washington (DC): United States Environmental Protection Agency. Available from: http://www.epa.gov/ncct/toxrefdb/, accessed 10 March 2015.

EPA (1991a). Second peer review of glyphosate. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-265.pdf, accessed 10 March 2015.

EPA (1991b). Glyphosate; 2-year combined chronic toxicity/carcinogenicity study in Sprague-Dawley rats - List A pesticide for reregistration. Document No. 008390. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-263.pdf, accessed June 2015; see also http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-268.pdf, accessed June 2015.

EPA (1991c). Peer review on glyphosate. Document No. 008527. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency.

EPA (1991d). Glyphosate - EPA registration No. 524-308 - 2-year chronic feeding/oncogenicity study in rats with technical glyphosate. Document No. 008897. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-268.pdf, accessed 10 March 2015.

EPA (1992). Determination of glyphosate in drinking water by direct-aqueous-injection HPLC, post column derivatization, and fluorescence detection. In: Methods for the determination of organic compounds in drinking water - Supplement II (EPA/600/R-92-129). Washington (DC): Environmental Monitoring Systems Laboratory, Office of Research and Development, United States Environmental Protection Agency. Available through NTIS (http://www.ntis.gov).

EPA (1993a). Reregistration Eligibility Decision (RED): Glyphosate. EPA 738-R-93-014. Washington (DC): Office of Prevention, Pesticides And Toxic Substances, Office of Pesticide Programs, United States Environmental Protection Agency. Available from: http://www.epa.gov/opp00001/chem_search/reg_actions/reregistration/red_PC-417300_1-Sep-93.pdf, accessed 10 March 2015.

EPA (1993b). RED facts: Glyphosate. EPA-738-F-93-011. Washington (DC): Office of Prevention, Pesticides, and Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/oppsrrd1/reregistration/REDs/factsheets/0178fact.pdf, accessed 4 May 2015.

EPA (1997). Pesticides industry sales and usage -1994 and 1995 market estimates. Washington (DC): Biological and Economic Analysis Division, Office of Pesticide Programs, Office of Prevention, Pesticides And Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/pestsales/95pestsales/market_estimates1995.pdf, accessed 10 March 2015.

EPA (2011). Pesticides industry sales and usage - 2006 and 2007 market estimates. Washington (DC): Biological and Economic Analysis Division, Office of Pesticide Programs, Office of Prevention, Pesticides And Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/opp00001/pestsales/07pestsales/market_estimates2007.pdf, accessed 10 March 2015.

EPA (2015). Toxicity Reference Database (ToxRefDB). Computational Toxicology Research Program, United States Environmental Protection Agency. Available from: http://www.epa.gov/ncct/toxrefdb/, accessed 10 March 2015.

Eriksson M, Hardell L, Carlberg M, Akerman M (2008). Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis. *Int J Cancer*, 123(7):1657-63. doi:10.1002/ijc.23589 PMID:18623080

European Commission (2002). Review report for the active substance glyphosate (6511/VI/99-final, 21 January 2002). Brussels: Health and Consumer Protection Directorate-General, European Commission. Available from: http://ec.europa.eu/food/plant/protection/evaluation/existactive/list1_glyphosate_en.pdf, accessed 29 April 2015.

Eustis SL, Hailey JR, Boorman GA, Haseman JK (1994). The utility of multiple-section sampling in the histopathological evaluation of the kidney for carcinogenicity studies. *Toxicol Pathol*, 22(5):457-72. doi:10.1177/019262339402200501 PMID:7899775

FAO (2000). Glyphosate, N-(phosphonomethyl)glycine. Specifications and evaluations for plant protection products. Rome: Food and Agriculture Organization of the United Nations. Available from: http://www.fao.

org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/glypho01.pdf, accessed 28 July 2015.

Farm Chemicals International (2015). Glyphosate. In: Crop Protection Database. Willoughby (OH): Meister Media Worldwide. Available from: http://www.farmchemicalsinternational.com/crop-protection-database/#/product/detail/203900/, accessed 2 February 2015.

Ferreira D, da Motta AC, Kreutz LC, Toni C, Loro VL, Barcellos LJ (2010). Assessment of oxidative stress in *Rhamdia quelen* exposed to agrichemicals. *Chemosphere*, 79(9):914–21. doi:10.1016/j.chemosphere.2010.03.024 PMID:20371099

Flower KB, Hoppin JA, Lynch CF, Blair A, Knott C, Shore DL *et al.* (2004). Cancer risk and parental pesticide application in children of Agricultural Health Study participants. *Environ Health Perspect*, 112(5):631–5. doi:10.1289/ehp.6586 PMID:15064173

Forgacs AL, Ding Q, Jaremba RG, Huhtaniemi IT, Rahman NA, Zacharewski TR (2012). BLTK1 murine Leydig cells: a novel steroidogenic model for evaluating the effects of reproductive and developmental toxicants. *Toxicol Sci*, 127(2):391–402. doi:10.1093/toxsci/kfs121 PMID:22461451

Freedonia (2012). World agricultural pesticides: industry study with forecasts for 2016 & 2021. Study #2902, August 2012. Cleveland (OH): The Freedonia Group. Available from: http://www.freedoniagroup.com/brochure/29xx/2902smwe.pdf, accessed 10 March 2015.

Frescura VD, Kuhn AW, Laughinghouse HD 4th, Paranhos JT, Tedesco SB (2013). Post-treatment with plant extracts used in Brazilian folk medicine caused a partial reversal of the antiproliferative effect of glyphosate in the *Allium cepa* test. *Biocell*, 37(2):23–8. PMID:24392578

Gasnier C, Benachour N, Clair E, Travert C, Langlois F, Laurant C *et al.* (2010). Digl protects against cell death provoked by glyphosate-based herbicides in human liver cell lines. *J Occup Med Toxicol*, 5(1):29 doi:10.1186/1745-6673-5-29 PMID:20979644

Gasnier C, Dumont C, Benachour N, Clair E, Chagnon MC, Séralini GE (2009). Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. *Toxicology*, 262(3):184–91. doi:10.1016/j.tox.2009.06.006 PMID:19539684

Gehin A, Guillaume YC, Millet J, Guyon C, Nicod L (2005). Vitamins C and E reverse effect of herbicide-induced toxicity on human epidermal cells HaCaT: a biochemometric approach. *Int J Pharm*, 288(2):219–26. doi:10.1016/j.ijpharm.2004.09.024 PMID:15620861

George J, Prasad S, Mahmood Z, Shukla Y (2010). Studies on glyphosate-induced carcinogenicity in mouse skin: a proteomic approach. *J Proteomics*, 73(5):951–64. doi:10.1016/j.jprot.2009.12.008 PMID:20045496

George J, Shukla Y (2013). Emptying of intracellular calcium pool and oxidative stress imbalance are associated with the glyphosate-induced proliferation in human skin keratinocytes HaCaT cells. *ISRN Dermatol*, 2013:825180 doi:10.1155/2013/825180 PMID:24073338

Geret F, Burgeot T, Haure J, Gagnaire B, Renault T, Communal PY *et al.* (2013). Effects of low-dose exposure to pesticide mixture on physiological responses of the Pacific oyster, Crassostrea gigas. *Environ Toxicol*, 28(12):689–99. doi:10.1002/tox.20764 PMID:22012874

Gholami-Seyedkolaei SJ, Mirvaghefi A, Farahmand H, Kosari AA, Gholami-Seyedkolaei SJ, Gholami-Seyedkolaei SJ (2013). Optimization of recovery patterns in common carp exposed to Roundup using response surface methodology: evaluation of neurotoxicity and genotoxicity effects and biochemical parameters. *Ecotoxicol Environ Saf*, 98:152–61. doi:10.1016/j.ecoenv.2013.09.009 PMID:24094415

Glusczak L, Loro VL, Pretto A, Moraes BS, Raabe A, Duarte MF *et al.* (2011). Acute exposure to glyphosate herbicide affects oxidative parameters in piava (*Leporinus obtusidens*). *Arch Environ Contam Toxicol*, 61(4):624–30. doi:10.1007/s00244-011-9652-4 PMID:21465245

Glyphosate Task Force (2014). How is glyphosate used? Glyphosate facts. Updated 10 March 2014. Darmstadt: Industry Task Force on Glyphosate. Available from: http://www.glyphosate.eu/how-glyphosate-used, accessed 21 April 2015.

Granby K, Vahl M (2001). Investigation of the herbicide glyphosate and the plant growth regulators chlormequat and mepiquat in cereals produced in Denmark. *Food Addit Contam*, 18(10):898–905. doi:10.1080/02652030119594 PMID:11569770

Greim H, Saltmiras D, Mostert V, Strupp C (2015). Evaluation of carcinogenic potential of the herbicide glyphosate, drawing on tumor incidence data from fourteen chronic/carcinogenicity rodent studies. *Crit Rev Toxicol*, 45(3):185–208. doi:10.3109/10408444.2014.1003423 PMID:25716480

Grisolia CK (2002). A comparison between mouse and fish micronucleus test using cyclophosphamide, mitomycin C and various pesticides. *Mutat Res*, 518(2):145–50. doi:10.1016/S1383-5718(02)00086-4 PMID:12113765

Guha N, Ward MH, Gunier R, Colt JS, Lea CS, Buffler PA *et al.* (2013). Characterization of residential pesticide use and chemical formulations through self-report and household inventory: the Northern California Childhood Leukemia study. *Environ Health Perspect*, 121(2):276–82. PMID:23110983

Gui YX, Fan XN, Wang HM, Wang G, Chen SD (2012). Glyphosate induced cell death through apoptotic and autophagic mechanisms. *Neurotoxicol Teratol*, 34(3):344–9. doi:10.1016/j.ntt.2012.03.005 PMID:22504123

Guilherme S, Gaivão I, Santos MA, Pacheco M (2010). European eel (*Anguilla anguilla*) genotoxic and

pro-oxidant responses following short-term exposure to Roundup-a glyphosate-based herbicide. *Mutagenesis*, 25(5):523–30. doi:10.1093/mutage/geq038 PMID:20643706

Guilherme S, Gaivão I, Santos MA, Pacheco M (2012a). DNA damage in fish (*Anguilla anguilla*) exposed to a glyphosate-based herbicide – elucidation of organ-specificity and the role of oxidative stress. *Mutat Res*, 743(1–2):1–9. doi:10.1016/j.mrgentox.2011.10.017 PMID:22266476

Guilherme S, Santos MA, Barroso C, Gaivão I, Pacheco M (2012b). Differential genotoxicity of Roundup(®) formulation and its constituents in blood cells of fish (*Anguilla anguilla*): considerations on chemical interactions and DNA damaging mechanisms. *Ecotoxicology*, 21(5):1381–90. doi:10.1007/s10646-012-0892-5 PMID:22526921

Guilherme S, Santos MA, Gaivão I, Pacheco M (2014a). Are DNA-damaging effects induced by herbicide formulations (Roundup® and Garlon®) in fish transient and reversible upon cessation of exposure? *Aquat Toxicol*, 155:213–21. doi:10.1016/j.aquatox.2014.06.007 PMID:25058560

Guilherme S, Santos MA, Gaivão I, Pacheco M (2014b). DNA and chromosomal damage induced in fish (*Anguilla anguilla* L.) by aminomethylphosphonic acid (AMPA)–the major environmental breakdown product of glyphosate. *Environ Sci Pollut Res Int*, 21(14):8730–9. doi:10.1007/s11356-014-2803-1 PMID:24696215

Hardell L, Eriksson M (1999). A case-control study of non-Hodgkin lymphoma and exposure to pesticides. *Cancer*, 85(6):1353–60. doi:10.1002/(SICI)1097-0142(19990315)85:6<1353::AID-CNCR19>3.0.CO;2-1 PMID:10189142

Hardell L, Eriksson M, Nordstrom M (2002). Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: pooled analysis of two Swedish case-control studies. *Leuk Lymphoma*, 43(5):1043–9. PMID:12148884

Hayes WJ Jr, Laws ER Jr editors. (1991). *Classes of pesticides*. Handbook of Pesticide Toxicology. Volume 3:. New York (NY): Academic Press, Inc.; p. 1340.

Heu C, Elie-Caille C, Mougey V, Launay S, Nicod L (2012). A step further toward glyphosate-induced epidermal cell death: involvement of mitochondrial and oxidative mechanisms. *Environ Toxicol Pharmacol*, 34(2):144–53. doi:10.1016/j.etap.2012.02.010 PMID:22522424

Hidalgo C, Rios C, Hidalgo M, Salvadó V, Sancho JV, Hernández F (2004). Improved coupled-column liquid chromatographic method for the determination of glyphosate and aminomethylphosphonic acid residues in environmental waters. *J Chromatogr A*, 1035(1):153–7. doi:10.1016/j.chroma.2004.02.044 PMID:15117086

Hilton CW (2012). Monsanto & the global glyphosate market: case study. The Wiglaf Journal. June 2012. Available from: http://www.wiglafjournal.com/pricing/2012/06/monsanto-the-global-glyphosate-market-case-study/, accessed 28 July 2015.

Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R *et al.* (1986). Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. *JAMA*, 256(9):1141–7. doi:10.1001/jama.1986.03380090081023 PMID:3801091

Humphries D, Byrtus G, Anderson AM (2005). Glyphosate residues in Alberta's atmospheric deposition, soils and surface waters. Alberta: Water Research Users Group, Alberta Environment. Available from: http://environment.gov.ab.ca/info/library/6444.pdf, accessed 13 November 2014.

IARC (2006). Data for the Monographs. In: Preamble to the IARC Monographs (amended January 2006). Lyon: International Agency for Research on Cancer. Available from: http://monographs.iarc.fr/ENG/Preamble/index.php, accessed 28 July 2015.

IARC (2014). Table 1. Key characteristics of carcinogens. In: Instructions for authors. Lyon: International Agency for Research on Cancer. Available from: http://monographs.iarc.fr/ENG/Preamble/previous/Instructions_to_Authors_S4.pdf, accessed 28 July 2015.

IPCS (1994). Glyphosate. Environmental Health Criteria 159. Geneva: International Programme on Chemical Safety, World Health Organization. Available from: http://www.inchem.org/documents/ehc/ehc/ehc159.htm, accessed 28 July 2015.

IPCS (1996). Glyphosate. WHO/FAO Data Sheets on Pesticides, No. 91 (WHO/PCS/DS/96.91). Geneva: International Programme on Chemical Safety, World Health Organization. Available from: http://apps.who.int/iris/handle/10665/63290.

IPCS (2005). Glyphosate. International Chemical Safety Card (ICSC 0160). Geneva: International Programme on Chemical Safety, World Health Organization. Available from: http://www.inchem.org/documents/icsc/icsc/eics0160.htm, accessed 2 February 2015.

Jacob GS, Garbow JR, Hallas LE, Kimack NM, Kishore GM, Schaefer J (1988). Metabolism of glyphosate in Pseudomonas sp. strain LBr. *Appl Environ Microbiol*, 54(12):2953–8. PMID:3223761

Jan MR, Shah J, Muhammad M, Ara B (2009). Glyphosate herbicide residue determination in samples of environmental importance using spectrophotometric method. *J Hazard Mater*, 169(1–3):742–5. doi:10.1016/j.jhazmat.2009.04.003 PMID:19411135

Jasper R, Locatelli GO, Pilati C, Locatelli C (2012). Evaluation of biochemical, hematological and oxidative parameters in mice exposed to the herbicide glyphosate-Roundup(®). *Interdiscip Toxicol*, 5(3):133–40. doi:10.2478/v10102-012-0022-5 PMID:23554553

Jauhiainen A, Räsänen K, Sarantila R, Nuutinen J, Kangas J (1991). Occupational exposure of forest workers to glyphosate during brush saw spraying work. *Am Ind Hyg*

Assoc J, 52(2):61–4. doi:10.1080/15298669191364334 PMID:2011980

JMPR (2006). Glyphosate. In: Joint FAO/WHO Meeting on Pesticide Residues. Pesticide residues in food – 2004: toxicological evaluations. Report No. WHO/PCS/06.1. Geneva: World Health Organization; pp. 95–169. Available from: http://whqlibdoc.who.int/publications/2006/9241665203_eng.pdf?ua=1, accessed 6 March 2015.

Johnson PD, Rimmer DA, Garrod AN, Helps JE, Mawdsley C (2005). Operator exposure when applying amenity herbicides by all-terrain vehicles and controlled droplet applicators. *Ann Occup Hyg*, 49(1):25–32. PMID:15596423

Kachuri L, Demers PA, Blair A, Spinelli JJ, Pahwa M, McLaughlin JR *et al.* (2013). Multiple pesticide exposures and the risk of multiple myeloma in Canadian men. *Int J Cancer*, 133(8):1846–58. doi:10.1002/ijc.28191 PMID:23564249

Kale PG, Petty BT Jr, Walker S, Ford JB, Dehkordi N, Tarasia S *et al.* (1995). Mutagenicity testing of nine herbicides and pesticides currently used in agriculture. *Environ Mol Mutagen*, 25(2):148–53. doi:10.1002/em.2850250208 PMID:7698107

Kalyanaraman B, Darley-Usmar V, Davies KJ, Dennery PA, Forman HJ, Grisham MB *et al.* (2012). Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations. *Free Radic Biol Med*, 52(1):1–6. doi:10.1016/j.freeradbiomed.2011.09.030 PMID:22027063

Karunanayake CP, Spinelli JJ, McLaughlin JR, Dosman JA, Pahwa P, McDuffie HH (2012). Hodgkin lymphoma and pesticides exposure in men: a Canadian case-control study. *J Agromed*, 17(1):30–9. doi:10.1080/1059924X.2012.632726 PMID:22191501

Kavlock R, Chandler K, Houck K, Hunter S, Judson R, Kleinstreuer N *et al.* (2012). Update on EPA's ToxCast program: providing high throughput decision support tools for chemical risk management. *Chem Res Toxicol*, 25(7):1287–302. doi:10.1021/tx3000939 PMID:22519603

Kaya B, Creus A, Yanikoğlu A, Cabré O, Marcos R (2000). Use of the Drosophila wing spot test in the genotoxicity testing of different herbicides. *Environ Mol Mutagen*, 36(1):40–6. doi:10.1002/1098-2280(2000)36:1<40::AID-EM6>3.0.CO;2-K PMID:10918358

Kier LD, Kirkland DJ (2013). Review of genotoxicity studies of glyphosate and glyphosate-based formulations. *Crit Rev Toxicol*, 43(4):283–315. doi:10.3109/10408444.2013.770820 PMID:23480780

Kim YH, Hong JR, Gil HW, Song HY, Hong SY (2013). Mixtures of glyphosate and surfactant TN20 accelerate cell death via mitochondrial damage-induced apoptosis and necrosis. *Toxicol In Vitro*, 27(1):191–7. doi:10.1016/j.tiv.2012.09.021 PMID:23099315

Kojima H, Katsura E, Takeuchi S, Niiyama K, Kobayashi K (2004). Screening for estrogen and androgen receptor activities in 200 pesticides by in vitro reporter gene assays using Chinese hamster ovary cells. *Environ Health Perspect*, 112(5):524–31. doi:10.1289/ehp.6649 PMID:15064155

Kojima H, Takeuchi S, Nagai T (2010). Endocrine-disrupting potential of pesticides via nuclear receptors and aryl hydrocarbon receptor *J Health Sci*, 56(4):374–86. doi:10.1248/jhs.56.374

Koller VJ, Fürhacker M, Nersesyan A, Mišík M, Eisenbauer M, Knasmueller S (2012). Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells. *Arch Toxicol*, 86(5):805–13. doi:10.1007/s00204-012-0804-8 PMID:22331240

Kolpin DW, Thurman EM, Lee EA, Meyer MT, Furlong ET, Glassmeyer ST (2006). Urban contributions of glyphosate and its degradate AMPA to streams in the United States. *Sci Total Environ*, 354(2–3):191–7. doi:10.1016/j.scitotenv.2005.01.028 PMID:16398995

Kreutz LC, Gil Barcellos LJ, de Faria Valle S, de Oliveira Silva T, Anziliero D, Davi dos Santos E *et al.* (2011). Altered hematological and immunological parameters in silver catfish (*Rhamdia quelen*) following short term exposure to sublethal concentration of glyphosate. *Fish Shellfish Immunol*, 30(1):51–7. doi:10.1016/j.fsi.2010.09.012 PMID:20883798

Kuang H, Wang L, Xu C (2011). Overview of analytical techniques for herbicides in foods. In: Soloneski S, Larramendy ML, editors. Herbicides, theory and applications. Available from: http://www.intechopen.com/books/herbicides-theory-and-applications, accessed 28 July 2015.

Kumar S, Khodoun M, Kettleson EM, McKnight C, Reponen T, Grinshpun SA *et al.* (2014). Glyphosate-rich air samples induce IL-33, TSLP and generate IL-13 dependent airway inflammation. *Toxicology*, 325:42–51. doi:10.1016/j.tox.2014.08.008 PMID:25172162

Kwiatkowska M, Huras B, Bukowska B (2014). The effect of metabolites and impurities of glyphosate on human erythrocytes (in vitro). *Pestic Biochem Physiol*, 109:34–43. doi:10.1016/j.pestbp.2014.01.003 PMID:24581382

Landgren O, Kyle RA, Hoppin JA, Beane Freeman LE, Cerhan JR, Katzmann JA *et al.* (2009). Pesticide exposure and risk of monoclonal gammopathy of undetermined significance in the Agricultural Health Study. *Blood*, 113(25):6386–91. doi:10.1182/blood-2009-02-203471 PMID:19387005

Larsen K, Najle R, Lifschitz A, Maté ML, Lanusse C, Virkel GL (2014). Effects of sublethal exposure to a glyphosate-based herbicide formulation on metabolic activities of different xenobiotic-metabolizing enzymes in rats. *Int J Toxicol*, 33(4):307–18. doi:10.1177/1091581814540481 PMID:24985121

Lavy TL, Cowell JE, Steinmetz JR, Massey JH (1992). Conifer seedling nursery worker exposure to

glyphosate. *Arch Environ Contam Toxicol*, 22(1):6–13. doi:10.1007/BF00213295 PMID:1554254

Lee EA, Strahan AP, Thurman EM (2001). Methods of analysis by the U.S. Geological Survey Organic Geochemistry Research Group — determination of glyphosate, aminomethylphophonic acid, and glufosinate in water using online solid-phase extraction and high-performance liquid chromatography/mass spectrometry. Open-File Report 01–454. Lawrence (KS): United States Geological Survey. Available from: http://ks.water.usgs.gov/pubs/reports/ofr.01-454.pdf, accessed 28 July 2015.

Lee WJ, Cantor KP, Berzofsky JA, Zahm SH, Blair A (2004a). Non-Hodgkin's lymphoma among asthmatics exposed to pesticides. *Int J Cancer*, 111(2):298–302. doi:10.1002/ijc.20273 PMID:15197786

Lee WJ, Colt JS, Heineman EF, McComb R, Weisenburger DD, Lijinsky W *et al.* (2005). Agricultural pesticide use and risk of glioma in Nebraska, United States. *Occup Environ Med*, 62(11):786–92. doi:10.1136/oem.2005.020230 PMID:16234405

Lee WJ, Lijinsky W, Heineman EF, Markin RS, Weisenburger DD, Ward MH (2004b). Agricultural pesticide use and adenocarcinomas of the stomach and oesophagus. *Occup Environ Med*, 61(9):743–9. doi:10.1136/oem.2003.011858 PMID:15317914

Lee WJ, Sandler DP, Blair A, Samanic C, Cross AJ, Alavanja MC (2007). Pesticide use and colorectal cancer risk in the Agricultural Health Study. *Int J Cancer*, 121(2):339–46. doi:10.1002/ijc.22635 PMID:17390374

Li AP, Long TJ (1988). An evaluation of the genotoxic potential of glyphosate. *Fundam Appl Toxicol*, 10(3):537–46. doi:10.1016/0272-0590(88)90300-4 PMID:3286348

Li Q, Lambrechts MJ, Zhang Q, Liu S, Ge D, Yin R *et al.* (2013). Glyphosate and AMPA inhibit cancer cell growth through inhibiting intracellular glycine synthesis. *Drug Des Dev Ther*, 7:635–43. PMID:23983455

Lioi MB, Scarfì MR, Santoro A, Barbieri R, Zeni O, Di Berardino D *et al.* (1998). Genotoxicity and oxidative stress induced by pesticide exposure in bovine lymphocyte cultures in vitro. *Mutat Res*, 403(1–2):13–20. doi:10.1016/S0027-5107(98)00010-4 PMID:9726001

Lopes FM, Varela Junior AS, Corcini CD, da Silva AC, Guazzelli VG, Tavares G *et al.* (2014). Effect of glyphosate on the sperm quality of zebrafish *Danio rerio*. *Aquat Toxicol*, 155:322–6. doi:10.1016/j.aquatox.2014.07.006 PMID:25089920

Lubick N (2009). Environmental impact of cocaine strategy assessed [News]*Nature*, Published online 12 November, doi doi:10.1038/news.2009.1080

Lueken A, Juhl-Strauss U, Krieger G, Witte I (2004). Synergistic DNA damage by oxidative stress (induced by H_2O_2) and nongenotoxic environmental chemicals in human fibroblasts. *Toxicol Lett*, 147(1):35–43. doi:10.1016/j.toxlet.2003.10.020 PMID:14700526

Lushchak OV, Kubrak OI, Storey JM, Storey KB, Lushchak VI (2009). Low toxic herbicide Roundup induces mild oxidative stress in goldfish tissues. *Chemosphere*, 76(7):932–7. doi:10.1016/j.chemosphere.2009.04.045 PMID:19450865

Mahendrakar K, Venkategowda PM, Rao SM, Mutkule DP (2014). Glyphosate surfactant herbicide poisoning and management. *Indian J Crit Care Med*, 18(5):328–30. doi:10.4103/0972-5229.132508 PMID:24914265

Malatesta M, Perdoni F, Santin G, Battistelli S, Muller S, Biggiogera M (2008). Hepatoma tissue culture (HTC) cells as a model for investigating the effects of low concentrations of herbicide on cell structure and function. *Toxicol In Vitro*, 22(8):1853–60. doi:10.1016/j.tiv.2008.09.006 PMID:18835430

Mañas F, Peralta L, Raviolo J, García Ovando H, Weyers A, Ugnia L *et al.* (2009b). Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests. *Ecotoxicol Environ Saf*, 72(3):834–7. doi:10.1016/j.ecoenv.2008.09.019 PMID:19013644

Mañas F, Peralta L, Raviolo J, Ovando HG, Weyers A, Ugnia L *et al.* (2009a). Genotoxicity of glyphosate assessed by the comet assay and cytogenetic tests. *Environ Toxicol Pharmacol*, 28(1):37–41. doi:10.1016/j.etap.2009.02.001 PMID:21783980

Mance D 3rd (2012). The great glyphosate debate. *Northern Woodlands* [online magazine]. 8 March. Available from: http://northernwoodlands.org/articles/article/the-great-glyphosate-debate, accessed 28 July 2015.

Mariager TP, Madsen PV, Ebbehøj NE, Schmidt B, Juhl A (2013). Severe adverse effects related to dermal exposure to a glyphosate-surfactant herbicide. *Clin Toxicol (Phila)*, 51(2):111–3. doi:10.3109/15563650.2013.763951 PMID:23360343

Marques A, Guilherme S, Gaivão I, Santos MA, Pacheco M (2014). Progression of DNA damage induced by a glyphosate-based herbicide in fish (*Anguilla anguilla*) upon exposure and post-exposure periods–insights into the mechanisms of genotoxicity and DNA repair. *Comp Biochem Physiol C Toxicol Pharmacol*, 166:126–33. doi:10.1016/j.cbpc.2014.07.009 PMID:25110831

Marques A, Guilherme S, Gaivão I, Santos MA, Pacheco M (2015). Erratum to: "Progression of DNA damage induced by a glyphosate-based herbicide in fish (*Anguilla anguilla*) upon exposure and post-exposure periods - Insights into the mechanisms of genotoxicity and DNA repair" [Comp. Biochem. Physiol. C 166 (2014) 126–133]. *Comp Biochem Physiol C Toxicol Pharmacol*, 168C:1 doi:10.1016/j.cbpc.2014.10.008 PMID:25521452

Martini CN, Gabrielli M, Vila MC (2012). A commercial formulation of glyphosate inhibits proliferation and differentiation to adipocytes and induces apoptosis in 3T3-L1 fibroblasts. *Toxicol In Vitro*, 26(6):1007–13. doi:10.1016/j.tiv.2012.04.017 PMID:22546541

McDuffie HH, Pahwa P, McLaughlin JR, Spinelli JJ, Fincham S, Dosman JA *et al.* (2001). Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health. *Cancer Epidemiol Biomarkers Prev*, 10(11):1155–63. PMID:11700263

McQueen H, Callan AC, Hinwood AL (2012). Estimating maternal and prenatal exposure to glyphosate in the community setting. *Int J Hyg Environ Health*, 215(6):570–6. doi:10.1016/j.ijheh.2011.12.002 PMID:22261298

Mesnage R, Bernay B, Séralini GE (2013). Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. *Toxicology*, 313(2–3):122–8. doi:10.1016/j.tox.2012.09.006 PMID:23000283

Meza-Joya FL, Ramírez-Pinilla MP, Fuentes-Lorenzo JL (2013). Toxic, cytotoxic, and genotoxic effects of a glyphosate formulation (Roundup® SL-Cosmoflux® 411F) in the direct-developing frog *Eleutherodactylus johnstonei*. *Environ Mol Mutagen*, 54(5):362–73. doi:10.1002/em.21775 PMID:23625742

Ministry of Chemicals & Fertilizers (2008). Performance of chemical & petrochemical industry at a glance (2001–2007). New Delhi: Monitoring and Evaluation Division, Department of Chemicals and Petrochemicals, Government of India. Available from: http://chemicals.nic.in/stat0107.pdf, accessed February 2015.

Mladinic M, Berend S, Vrdoljak AL, Kopjar N, Radic B, Zeljezic D (2009b). Evaluation of genome damage and its relation to oxidative stress induced by glyphosate in human lymphocytes in vitro. *Environ Mol Mutagen*, 50(9):800–7. doi:10.1002/em.20495 PMID:19402152

Mladinic M, Perkovic P, Zeljezic D (2009a). Characterization of chromatin instabilities induced by glyphosate, terbuthylazine and carbofuran using cytome FISH assay. *Toxicol Lett*, 189(2):130–7. doi:10.1016/j.toxlet.2009.05.012 PMID:19477249

MLHB (2013). Determination of glyphosate residues in human urine samples from 18 European countries. Bremen: Medical Laboratory of Bremen. Available from: https://www.foeeurope.org/sites/default/files/glyphosate_studyresults_june12.pdf, accessed 24 November 2014.

Modesto KA, Martinez CB (2010a). Effects of Roundup Transorb on fish: hematology, antioxidant defenses and acetylcholinesterase activity. *Chemosphere*, 81(6):781–7. doi:10.1016/j.chemosphere.2010.07.005 PMID:20684975

Modesto KA, Martinez CB (2010b). Roundup causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish *Prochilodus lineatus*. *Chemosphere*, 78(3):294–9. doi:10.1016/j.chemosphere.2009.10.047 PMID:19910015

Mohamed AH (2011). Sublethal toxicity of Roundup to immunological and molecular aspects of *Biomphalaria alexandrina* to *Schistosoma mansoni* infection. *Ecotoxicol Environ Saf*, 74(4):754–60. doi:10.1016/j.ecoenv.2010.10.037 PMID:21126764

Monge P, Wesseling C, Guardado J, Lundberg I, Ahlbom A, Cantor KP *et al.* (2007). Parental occupational exposure to pesticides and the risk of childhood leukemia in Costa Rica. *Scand J Work Environ Health*, 33(4):293–303. doi:10.5271/sjweh.1146 PMID:17717622

Monroy CM, Cortés AC, Sicard DM, de Restrepo HG (2005). [Cytotoxicity and genotoxicity of human cells exposed in vitro to glyphosate] *Biomedica*, 25(3):335–45. doi:10.7705/biomedica.v25i3.1358 PMID:16276681

Moreno NC, Sofia SH, Martinez CB (2014). Genotoxic effects of the herbicide Roundup Transorb and its active ingredient glyphosate on the fish *Prochilodus lineatus*. *Environ Toxicol Pharmacol*, 37(1):448–54. doi:10.1016/j.etap.2013.12.012 PMID:24448465

Mortensen OS, Sørensen FW, Gregersen M, Jensen K (2000). [Poisonings with the herbicides glyphosate and glyphosate-trimesium] [in Danish] *Ugeskr Laeger*, 162(35):4656–9. PMID:10986892

Motojyuku M, Saito T, Akieda K, Otsuka H, Yamamoto I, Inokuchi S (2008). Determination of glyphosate, glyphosate metabolites, and glufosinate in human serum by gas chromatography-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*, 875(2):509–14. doi:10.1016/j.jchromb.2008.10.003 PMID:18945648

Muangphra P, Kwankua W, Gooneratne R (2014). Genotoxic effects of glyphosate or paraquat on earthworm coelomocytes. *Environ Toxicol*, 29(6):612–20. doi:10.1002/tox.21787 PMID:22644885

Nakashima K, Yoshimura T, Mori H, Kawaguchi M, Adachi S, Nakao T *et al.* (2002). [Effects of pesticides on cytokine production by human peripheral blood mononuclear cells–fenitrothion and glyphosate] *Chudoku Kenkyu*, 15(2):159–65. PMID:12108020

NCBI (2015). Glyphosate. Compound summary for CID 3496. PubChem Open Chemistry Database. Bethesda (MD): National Center for Biotechnology Information, United States National Library of Medicine. Available from: http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=3496, accessed 5 March 2015.

Nedelkoska TV, Low GKC (2004). High-performance liquid chromatographic determination of glyphosate in water and plant material after pre-column derivatisation with 9-fluorenylmethyl chloroformate. *Anal Chim Acta*, 511(1):145–53. doi:10.1016/j.aca.2004.01.027

NIH (2015). Questionnaires and study data. Agricultural Health Study. National Institutes of Health. Available from: http://aghealth.nih.gov/collaboration/questionnaires.html, accessed 12 June 2015.

Nordström M, Hardell L, Magnuson A, Hagberg H, Rask-Andersen A (1998). Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukaemia evaluated in a case-control study. *Br*

J Cancer, 77(11):2048–52. doi:10.1038/bjc.1998.341 PMID:9667691

NPIC (2010). Glyphosate. General fact sheet. Oregon State University: National Pesticide Information Center. Available from: http://npic.orst.edu/factsheets/glyphogen.pdf, accessed June 2015.

Nwani CD, Nagpure NS, Kumar R, Kushwaha B, Lakra WS (2013). DNA damage and oxidative stress modulatory effects of glyphosate-based herbicide in freshwater fish, *Channa punctatus*. *Environ Toxicol Pharmacol*, 36(2):539–47. doi:10.1016/j.etap.2013.06.001 PMID:23816461

Omran NE, Salama WM (2013). The endocrine disrupter effect of atrazine and glyphosate on *Biomphalaria alexandrina* snails. *Toxicol Ind Health*, doi:10.1177/0748233713506959 PMID:24215068

Orsi L, Delabre L, Monnereau A, Delval P, Berthou C, Fenaux P *et al.* (2009). Occupational exposure to pesticides and lymphoid neoplasms among men: results of a French case-control study. *Occup Environ Med*, 66(5):291–8. doi:10.1136/oem.2008.040972 PMID:19017688

Ortiz-Ordoñez E, Uría-Galicia E, Ruiz-Picos RA, Duran AG, Trejo YH, Sedeño-Díaz JE *et al.* (2011). Effect of Yerbimat herbicide on lipid peroxidation, catalase activity, and histological damage in gills and liver of the freshwater fish *Goodea atripinnis*. *Arch Environ Contam Toxicol*, 61(3):443–52. doi:10.1007/s00244-011-9648-0 PMID:21305274

Paganelli A, Gnazzo V, Acosta H, López SL, Carrasco AE (2010). Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signalling. *Chem Res Toxicol*, 23(10):1586–95. doi:10.1021/tx1001749 PMID:20695457

Pahwa P, Karunanayake CP, Dosman JA, Spinelli JJ, McLaughlin JR; Cross-Canada Group(2011). Soft-tissue sarcoma and pesticides exposure in men: results of a Canadian case-control study. *J Occup Environ Med*, 53(11):1279–86. doi:10.1097/JOM.0b013e3182307845 PMID:22068131

Park JS, Kwak SJ, Gil HW, Kim SY, Hong SY (2013). Glufosinate herbicide intoxication causing unconsciousness, convulsion, and 6th cranial nerve palsy. *J Korean Med Sci*, 28(11):1687–9. doi:10.3346/jkms.2013.28.11.1687 PMID:24265537

Paz-y-Miño C, Muñoz MJ, Maldonado A, Valladares C, Cumbal N, Herrera C *et al.* (2011). Baseline determination in social, health, and genetic areas in communities affected by glyphosate aerial spraying on the northeastern Ecuadorian border. *Rev Environ Health*, 26(1):45–51. doi:10.1515/reveh.2011.007 PMID:21714381

Paz-y-Miño C, Sánchez ME, Aréval M, Muñoz MJ, Witte T, De-la-Carrera GO *et al.* (2007). Evaluation of DNA damage in an Ecuadorian population exposed to glyphosate. *Genet Mol Biol*, 30(2):456–60. doi:10.1590/S1415-47572007000300026

Peluso M, Munnia A, Bolognesi C, Parodi S (1998). ^{32}P-postlabeling detection of DNA adducts in mice treated with the herbicide Roundup. *Environ Mol Mutagen*, 31(1):55–9. doi:10.1002/(SICI)1098-2280(1998)31:1<55::AID-EM8>3.0.CO;2-A PMID:9464316

Perry L, Adams RD, Bennett AR, Lupton DJ, Jackson G, Good AM *et al.* (2014). National toxicovigilance for pesticide exposures resulting in health care contact - An example from the UK's National Poisons Information Service. *Clin Toxicol (Phila)*, 52(5):549–55. doi:10.3109/15563650.2014.908203 PMID:24735003

Pesticide Residues Committee (2007). Pesticide residues monitoring report. Fourth quarter report 2006. York: Pesticide Residues Committee. Available from: http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/PRiF/PRC-Pesticides-Residues-Commitee/PRC_Results_and_Reports/PRC_Reports_by_Year/pesticide-residue-committee-prc-2006, accessed 2 November 2014.

Pesticide Residues Committee (2008). Pesticide residues monitoring report. Fourth quarter report 2007. York: Pesticide Residues Committee. Available from: http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/PRiF/PRC-Pesticides-Residues-Commitee/PRC_Results_and_Reports/PRC_Reports_by_Year/pesticides-residues-committee-prc-reports-2007, accessed 2 November 2014.

Pesticide Residues Committee (2009). Pesticide residues monitoring report. Fourth quarter report 2008. York: Pesticide Residues Committee. Available from: http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/PRiF/PRC-Pesticides-Residues-Commitee/PRC_Results_and_Reports/PRC_Reports_by_Year/pesticide-residues-committee-prc-reports-2009.htm?wbc_purpose=Ba, accessed 2 November 2014.

Pesticide Residues Committee (2010). Pesticide residues monitoring report. Fourth quarter report 2009. York: Pesticide Residues Committee. Available from: http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/PRiF/PRC-Pesticides-Residues-Commitee/PRC_Results_and_Reports/PRC_Reports_by_Year/pesticide-residues-committee-prc-reports-2010, accessed 2 November 2014.

Piola L, Fuchs J, Oneto ML, Basack S, Kesten E, Casabé N (2013). Comparative toxicity of two glyphosate-based formulations to *Eisenia andrei* under laboratory conditions. *Chemosphere*, 91(4):545–51. doi:10.1016/j.chemosphere.2012.12.036 PMID:23332878

Poletta GL, Kleinsorge E, Paonessa A, Mudry MD, Larriera A, Siroski PA (2011). Genetic, enzymatic

and developmental alterations observed in *Caiman latirostris* exposed in ovo to pesticide formulations and mixtures in an experiment simulating environmental exposure. *Ecotoxicol Environ Saf*, 74(4):852–9. doi:10.1016/j.ecoenv.2010.12.005 PMID:21185601

Poletta GL, Larriera A, Kleinsorge E, Mudry MD (2009). Genotoxicity of the herbicide formulation Roundup (glyphosate) in broad-snouted caiman (*Caiman latirostris*) evidenced by the Comet assay and the Micronucleus test. *Mutat Res*, 672(2):95–102. doi:10.1016/j.mrgentox.2008.10.007 PMID:19022394

Prasad S, Srivastava S, Singh M, Shukla Y (2009). Clastogenic effects of glyphosate in bone marrow cells of swiss albino mice. *J Toxicol*, 2009:308985 doi:10.1155/2009/308985 PMID:20107585

Rank J, Jensen AG, Skov B, Pedersen LH, Jensen K (1993). Genotoxicity testing of the herbicide Roundup and its active ingredient glyphosate isopropylamine using the mouse bone marrow micronucleus test, *Salmonella* mutagenicity test, and *Allium* anaphase-telophase test. *Mutat Res*, 300(1):29–36. doi:10.1016/0165-1218(93)90136-2 PMID:7683765

República de El Salvador (2013). Asamblea Legislativa aprueba reformas que prohíben pesticidas que dañan la salud, 5 September 2013. Available from: http://www.asamblea.gob.sv/noticias/archivo-de-noticias/asamblea-legislativa-aprueba-reformas-que-prohiben-pesticidas-que-danan-la-salud, accessed 28 April 2015. [Spanish]

Richard S, Moslemi S, Sipahutar H, Benachour N, Seralini GE (2005). Differential effects of glyphosate and Roundup on human placental cells and aromatase. *Environ Health Perspect*, 113(6):716–20. doi:10.1289/ehp.7728 PMID:15929894

Roberts DM, Buckley NA, Mohamed F, Eddleston M, Goldstein DA, Mehrsheikh A *et al.* (2010). A prospective observational study of the clinical toxicology of glyphosate-containing herbicides in adults with acute self-poisoning. *Clin Toxicol (Phila)*, 48(2):129–36. doi:10.3109/15563650903476491 PMID:20136481

Roustan A, Aye M, De Meo M, Di Giorgio C (2014). Genotoxicity of mixtures of glyphosate and atrazine and their environmental transformation products before and after photoactivation. *Chemosphere*, 108:93–100. doi:10.1016/j.chemosphere.2014.02.079 PMID:24875917

Ruder AM, Waters MA, Butler MA, Carreón T, Calvert GM, Davis-King KE *et al.*; Brain Cancer Collaborative Study Group(2004). Gliomas and farm pesticide exposure in men: the Upper Midwest Health Study. *Arch Environ Health*, 59(12):650–7. doi:10.1080/00039890409602949 PMID:16789473

Rueppel ML, Brightwell BB, Schaefer J, Marvel JT (1977). Metabolism and degradation of glyphosphate in soil and water. *J Agric Food Chem*, 25(3):517–28. doi:10.1021/jf60211a018 PMID:858844

Rumack BH (2015). Emergency medical treatment. Glyphosate isopropylamine salt. POISINDEX(R) Information System. CCIS Volume 164, edition expires May, 2015. Available from: http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~M2Dk5e:2.

Sanchís J, Kantiani L, Llorca M, Rubio F, Ginebreda A, Fraile J *et al.* (2012). Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry. *Anal Bioanal Chem*, 402(7):2335–45. doi:10.1007/s00216-011-5541-y PMID:22101424

Schinasi L, Leon ME (2014). Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: a systematic review and meta-analysis. *Int J Environ Res Public Health*, 11(4):4449–527. doi:10.3390/ijerph110404449 PMID:24762670

Séralini GE, Clair E, Mesnage R, Gress S, Defarge N, Manuela Malatesta M *et al.* (2014). Republished study:long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Environmental Sciences Europe*, 26(1):1–14. doi:10.1186/s12302-014-0014-5

Siddiqui S, Meghvansi MK, Khan SS (2012). Glyphosate, alachor and maleic hydrazide have genotoxic effect on *Trigonella foenum-graecum* L. *Bull Environ Contam Toxicol*, 88(5):659–65. doi:10.1007/s00128-012-0570-6 PMID:22392005

Simonsen L, Fomsgaard IS, Svensmark B, Spliid NH (2008). Fate and availability of glyphosate and AMPA in agricultural soil. *J Environ Sci Health B*, 43(5):365–75. doi:10.1080/03601230802062000 PMID:18576216

Sinhorin VD, Sinhorin AP, Teixeira JM, Miléski KM, Hansen PC, Moreira PS *et al.* (2014). Effects of the acute exposition to glyphosate-based herbicide on oxidative stress parameters and antioxidant responses in a hybrid Amazon fish surubim (*Pseudoplatystoma* sp). *Ecotoxicol Environ Saf*, 106:181–7. doi:10.1016/j.ecoenv.2014.04.040 PMID:24840881

Siviková K, Dianovský J (2006). Cytogenetic effect of technical glyphosate on cultivated bovine peripheral lymphocytes *Int J Hyg Environ Health*, 209(1):15–20. doi:10.1016/j.ijheh.2005.07.005 PMID:16373198

Slaninova A, Smutna M, Modra H, Svobodova Z (2009). A review: oxidative stress in fish induced by pesticides. *Neuro Endocrinol Lett*, 30:Suppl 1: 2–12. PMID:20027135

Solomon KR, Anadón A, Carrasquilla G, Cerdeira AL, Marshall J, Sanin LH (2007). Coca and poppy eradication in Colombia: environmental and human health assessment of aerially applied glyphosate. *Rev Environ Contam Toxicol*, 190:43–125. doi:10.1007/978-0-387-36903-7_2 PMID:17432331

Sorahan T (2015). Multiple myeloma and glyphosate use: a re-analysis of US Agricultural Health Study (AHS) data. *Int J Environ Res Public Health*, 12(2):1548–59. doi:10.3390/ijerph120201548 PMID:25635915

Sørensen FW, Gregersen M (1999). Rapid lethal intoxication caused by the herbicide glyphosate-trimesium (Touchdown). *Hum Exp Toxicol*, 18(12):735–7. doi:10.1191/096032799678839590 PMID:10627661

Sribanditmongkol P, Jutavijittum P, Pongraveevongsa P, Wunnapuk K, Durongkadech P (2012). Pathological and toxicological findings in glyphosate-surfactant herbicide fatality: a case report. *Am J Forensic Med Pathol*, 33(3):234–7. doi:10.1097/PAF.0b013e31824b936c PMID:22835958

Stella J, Ryan M (2004). Glyphosate herbicide formulation: a potentially lethal ingestion. *Emerg Med Australas*, 16(3):235–9. doi:10.1111/j.1742-6723.2004.00593.x PMID:15228468

Székács A, Darvas B (2012). Forty years with glyphosate. In: Hasaneen MNAE-G, editor. Herbicides – properties, synthesis and control of weeds. Croatia: InTech, pp. 247–84. Available from: http://cdn.intechweb.org/pdfs/25624.pdf, accessed 28 July 2015.

Takeuchi S, Iida M, Yabushita H, Matsuda T, Kojima H (2008). In vitro screening for aryl hydrocarbon receptor agonistic activity in 200 pesticides using a highly sensitive reporter cell line, DR-EcoScreen cells, and in vivo mouse liver cytochrome P450-1A induction by propanil, diuron and linuron. *Chemosphere*, 74(1):155–65. doi:10.1016/j.chemosphere.2008.08.015 PMID:18835618

Temple WA, Smith NA (1992). Glyphosate herbicide poisoning experience in New Zealand. *N Z Med J*, 105(933):173–4. PMID:1589162

Thongprakaisang S, Thiantanawat A, Rangkadilok N, Suriyo T, Satayavivad J (2013). Glyphosate induces human breast cancer cells growth via estrogen receptors. *Food Chem Toxicol*, 59:129–36. doi:10.1016/j.fct.2013.05.057 PMID:23756170

Tian J, Shi H, Li X, Yin Y, Chen L (2012). Coupling mass balance analysis and multi-criteria ranking to assess the commercial-scale synthetic alternatives: a case study on glyphosate. *Green Chem*, 14:1990–2000.

Tice RR, Austin CP, Kavlock RJ, Bucher JR (2013). Improving the human hazard characterization of chemicals: a Tox21 update. *Environ Health Perspect*, 121(7):756–65. doi:10.1289/ehp.1205784 PMID:23603828

Tomlin CDS, editor (2000). The pesticide manual: a world compendium. 12th ed. Croydon: British Crop Protection Council. Available from: http://trove.nla.gov.au/work/6273016, accessed 28 July 2015.

Transparency Market Research (2014). Global glyphosate market expected to reach US$8.79 billion in 2019. New York: Transparency Market Research. Posted on 9 December 2014. Available from: http://www.transparencymarketresearch.com/pressrelease/glyphosate-market.htm, accessed 21 April 2015.

Truta E, Vochita G, Rosu CM, Zamfirache MM, Olteanu Z (2011). Evaluation of Roundup-induced toxicity on genetic material and on length growth of barley seedlings. *Acta Biol Hung*, 62(3):290–301. doi:10.1556/ABiol.62.2011.3.8 PMID:21840831

Tu M, Hurd C, Randall JM (2001). Weed control methods handbook: tools & techniques for use in natural areas. Version April 2001. Arlington (VA): Wildland Invasive Species Team, The Nature Conservancy. Available from: http://www.invasive.org/gist/products/handbook/01.TitleContents.pdf, accessed 28 July 2015.

Uren Webster TM, Laing LV, Florance H, Santos EM (2014). Effects of glyphosate and its formulation, Roundup, on reproduction in zebrafish (*Danio rerio*). *Environ Sci Technol*, 48(2):1271–9. doi:10.1021/es404258h PMID:24364672

Vainio H, Linnainmaa K, Kähönen M, Nickels J, Hietanen E, Marniemi J *et al.* (1983). Hypolipidemia and peroxisome proliferation induced by phenoxyacetic acid herbicides in rats. *Biochem Pharmacol*, 32(18):2775–9. doi:10.1016/0006-2952(83)90091-6 PMID:6626247

Varona M, Henao GL, Díaz S, Lancheros A, Murcia A, Rodríguez N *et al.* (2009). Evaluación de los efectos del glifosato y otros plaguicidas en la salud humana en zonas objeto del programa de erradicación de cultivos ilícitos. [Effects of aerial applications of the herbicide glyphosate and insecticides on human health] *Biomedica*, 29(3):456–75. [Spanish]. doi:10.7705/biomedica.v29i3.16 PMID:20436997

Vasiluk L, Pinto LJ, Moore MM (2005). Oral bioavailability of glyphosate: studies using two intestinal cell lines. *Environ Toxicol Chem*, 24(1):153–60. doi:10.1897/04-088R.1 PMID:15683179

Vera-Candioti J, Soloneski S, Larramendy ML (2013). Evaluation of the genotoxic and cytotoxic effects of glyphosate-based herbicides in the ten spotted livebearer fish *Cnesterodon decemmaculatus* (Jenyns, 1842). *Ecotoxicol Environ Saf*, 89:166–73. doi:10.1016/j.ecoenv.2012.11.028 PMID:23273868

Vigfusson NV, Vyse ER (1980). The effect of the pesticides, Dexon, Captan and Roundup, on sister-chromatid exchanges in human lymphocytes in vitro. *Mutat Res*, 79(1):53–7. doi:10.1016/0165-1218(80)90147-0 PMID:7432366

Waddell BL, Zahm SH, Baris D, Weisenburger DD, Holmes F, Burmeister LF *et al.* (2001). Agricultural use of organophosphate pesticides and the risk of non-Hodgkin's lymphoma among male farmers (United States). *Cancer Causes Control*, 12(6):509–17. doi:10.1023/A:1011293208949 PMID:11519759

Walsh LP, McCormick C, Martin C, Stocco DM (2000). Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression.

Environ Health Perspect, 108(8):769–76. doi:10.1289/ehp.00108769 PMID:10964798

Wang G, Deng S, Li C, Liu Y, Chen L, Hu C (2012). Damage to DNA caused by UV-B radiation in the desert cyanobacterium *Scytonema javanicum* and the effects of exogenous chemicals on the process. *Chemosphere*, 88(4):413–7. doi:10.1016/j.chemosphere.2012.02.056 PMID:22436589

Wester RC, Melendres J, Sarason R, McMaster J, Maibach HI (1991). Glyphosate skin binding, absorption, residual tissue distribution, and skin decontamination. *Fundam Appl Toxicol*, 16(4):725–32. doi:10.1016/0272-0590(91)90158-Z PMID:1884912

Xie L, Thrippleton K, Irwin MA, Siemering GS, Mekebri A, Crane D *et al.* (2005). Evaluation of estrogenic activities of aquatic herbicides and surfactants using an rainbow trout vitellogenin assay. *Toxicol Sci*, 87(2):391–8. doi:10.1093/toxsci/kfi249 PMID:16049272

Yadav SS, Giri S, Singha U, Boro F, Giri A (2013). Toxic and genotoxic effects of Roundup on tadpoles of the Indian skittering frog (*Euflictis cyanophlyctis*) in the presence and absence of predator stress. *Aquat Toxicol*, 132–133:1–8. doi:10.1016/j.aquatox.2013.01.016 PMID:23454306

Yin G (2011). Glyphosate: There is no substitute. Farm Chemicals International. 3 March 2011. Willoughby (OH): Meister Media Worldwide. Available from: http://www.farmchemicalsinternational.com/crop-inputs/herbicides/glyphosate-there-is-no-substitute/, accessed June 2015.

Yoshioka N, Asano M, Kuse A, Mitsuhashi T, Nagasaki Y, Ueno Y (2011). Rapid determination of glyphosate, glufosinate, bialaphos, and their major metabolites in serum by liquid chromatography-tandem mass spectrometry using hydrophilic interaction chromatography. *J Chromatogr A*, 1218(23):3675–80. doi:10.1016/j.chroma.2011.04.021 PMID:21530973

Yue Y, Zhang Y, Zhou L, Qin J, Chen X (2008). In vitro study on the binding of herbicide glyphosate to human serum albumin by optical spectroscopy and molecular modeling. *J Photochem Photobiol B*, 90(1):26–32. doi:10.1016/j.jphotobiol.2007.10.003 PMID:18035550

Zahm SH, Weisenburger DD, Babbitt PA, Saal RC, Vaught JB, Cantor KP *et al.* (1990). A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. *Epidemiology*, 1(5):349–56. doi:10.1097/00001648-199009000-00004 PMID:2078610

Zhao W, Yu H, Zhang J, Shu L (2013). [Effects of glyphosate on apoptosis and expressions of androgen-binding protein and vimentin mRNA in mouse Sertoli cells] *Nan Fang Yi Ke Da Xue Xue Bao*, 33(11):1709–13. PMID:24273285

Zouaoui K, Dulaurent S, Gaulier JM, Moesch C, Lachâtre G (2013). Determination of glyphosate and AMPA in blood and urine from humans: about 13 cases of acute intoxication. *Forensic Sci Int*, 226(1–3):e20–5. doi:10.1016/j.forsciint.2012.12.010 PMID:23291146

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Toxicokinetics

- **Absorption:** No direct human study of absorption of glyphosate was available to the working group; however, several studies in agricultural applicators reported detectable levels of glyphosate in urine (Acquavella et al., 2004) (Curwin et al., 2007), indicative of absorption. In rodents, several studies showed 30-40% absorption after administration of a single oral dose (Brewster *et al.*, 1991) (Chan & Mahler, 1992) (Williams *et al.*, 2000). In a repeat-dose study, ~15% of glyphosate was found to be absorbed (Williams *et al.*, 2000).
- **Distribution:** No data on systemic tissue distribution of glyphosate in humans were available to the working group. In a rat study, the $t_{1/2}$ of glyphosate in plasma was estimated at 33 hours (Bernal *et al.*, 2010). In the sub-chronic 14-days feeding study in rats, glyphosate reached steady-state levels in blood by 6 days (Williams et al., 2000) and the concentrations in tissues had the following rank order: kidneys > spleen > fat > liver. Repeat administration had no effect on distribution of glyphosate (Williams et al., 2000).
- **Metabolism:** In the environment, glyphosate is degraded by soil microbes, primarily to aminomethylphosphoric acid (AMPA) and carbon dioxide (Jacob *et al.*, 1988). Glyphosate is not well metabolized in humans or other mammals. In rats, small amounts of AMPA were detected in plasma (Bernal et al., 2010) and in colon (Brewster et al., 1991); with the latter being attributed to intestinal microbial metabolism. In humans, small amounts of AMPA are detectable in blood in cases of deliberate glyphosate poisoning (Motojyuku et al., 2008). Few studies examined possible effects of glyphosate on metabolizing enzymes and no firm conclusions can be drawn.
- **Excretion:** Studies in rodents showed that systemically absorbed glyphosate is excreted unchanged into urine and the greatest amount is excreted in feces indicating poor absorption. Glyphosate was detected in urine of humans occupationally exposed to glyphosate (Acquavella et al., 2004) (Curwin et al., 2007).

Key characteristics

- **Electrophilicity:** Glyphosate is not electrophilic and is not metabolized to an electrophile.
- **Genotoxicity:** In vivo evidence on genotoxicity of glyphosate is largely inconsistent in studies in rodents and no conclusions can be drawn from human studies due to mixed exposures to pesticides and other chemicals. In vitro data in human and animal cells contains some evidence of genotoxicity of glyphosate and AMPA; however, a number of studies failed to observe evidence for genotoxicity. Positive studies for glyphosate, AMPA and commercial formulations of glyphosate are available in a variety of plants, fish and other marine organisms. The majority of standard Ames test bacterial strains were not affected by glyphosate or AMPA, even in presence of metabolic activation.
- **Altered Repair Genomic Instability:** No data.
- **Chronic Inflammation or Oxidative Stress:** Strong evidence exists that glyphosate, AMPA and commercial formulations of glyphosate can induce oxidative stress in many rodent tissues in vivo and in rodent and human cells in vitro. Similar findings have been reported in fish and other aquatic species. Various endpoints (lipid peroxidation markers, oxidative DNA adducts, dysregulation of antioxidant enzymes, etc.) have been evaluated across numerous studies which increases confidence in the overall database. It is yet to be determined, however, the exact mechanism of such effects.
- **Receptor Mediated:** Glyphosate was not found to be a ligand to a number of xenobiotic metabolism-inducing nuclear receptors (AhR, PPARs, PXR); however, some studies suggested that it may act as an agonist and antagonist to hormone receptors, ER and AR. Given the paucity of the available data, insofar the compound used in these studies (glyphosate, or various

commercial formulations of the pesticide and combinations thereof), it is difficult to ascertain whether the observed effects are due to glyphosate or other substances.

- **Proliferation or Death:** A number of studies in human and rodent cell lines have observed cytotoxicity and cell death, attributed to the apoptosis pathway, in high micro-molar concentrations or greater. Some studies examined the effects of glyphosate alone in comparison to mixtures of glyphosate with adjuvants to mimic commercial formulations, and found that adjuvants generally exacerbated effects of glyphosate.
- **Immunosuppression:** There is some evidence that glyphosate may affect the immune system, both humoral and cellular response, upon chronic treatment in rodents. Several studies in fish, both using commercial formulations of glyphosate rather than the pure chemical, also reported immunosuppressive effects.
- **Epigentic effects:** No data
- **Immortalization:** No data.
- **Other:** None

Toxicity confirming target tissue/site: to be filled in once target tissues are confirmed

Susceptibility: No data

Additional relevant data: No data

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Biomonitoring of Genotoxic Risk in Agricultural Workers from Five Colombian Regions: Association to Occupational Exposure to Glyphosate

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In order to assess possible human effects associated with glyphosate formulations used in the Colombian aerial spray program for control of illicit crops, a cytogenetic biomonitoring study was carried out in subjects from five Colombian regions, characterized by different exposure to glyphosate and other pesticides. Women of reproductive age (137 persons 15–49 yr old) and their spouses (137 persons) were interviewed to obtain data on current health status, history, lifestyle, including past and current occupational exposure to pesticides, and factors including those known to be associated with increased frequency of micronuclei (MN). In regions where glyphosate was being sprayed, blood samples were taken prior to spraying (indicative of baseline exposure), 5 d after spraying, and 4 mo after spraying. Lymphocytes were cultured and a cytokinesis-block micronucleus cytome assay was applied to evaluate chromosomal damage and cytotoxicity. Compared with Santa Marta, where organic coffee is grown without pesticides, the baseline frequency of binucleated cells with micronuclei (BNMN) was significantly greater in subjects from the other four regions. The highest frequency of BNMN was in Boyacá, where no aerial eradication spraying of glyphosate was conducted, and in Valle del Cauca, where glyphosate was used for maturation of sugar cane. Region, gender, and older age (≥35 yr) were the only variables associated with the frequency of BNMN measured before spraying. A significant increase in frequency of BNMN between first and second sampling was observed in Nariño, Putumayo, and Valle immediately (<5 d) after spraying. In the post-spray sample, those who reported direct contact with the eradication spray showed a higher quantitative frequency of BNMN compared to those without glyphosate exposure. The increase in frequency of BNMN observed immediately after the glyphosate spraying was not consistent with the rates of application used in the regions and there was no association between self-reported direct contact with eradication sprays and frequency of BNMN. Four months after spraying, a statistically significant decrease in the mean frequency of BNMN compared with the second sampling was observed in Nariño, but not in Putumayo and Valle del Cauca. Overall, data suggest that genotoxic damage associated with glyphosate spraying for control of illicit crops as evidenced by MN test is small and appears to be transient. Evidence indicates that the genotoxic risk potentially associated with exposure to glyphosate in the areas where the herbicide is applied for coca and poppy eradication is low.

Glyphosate (*N*-phosphonomethyl glycine), a nonselective herbicide, is the active ingredient of a number of herbicide formulations and one of the most widely used pesticides on a global basis (Baylis, 2000; Woodburn, 2000; Duke & Powles, 2008). It is a postemergence herbicide, effective for the control of annual, biennial, and perennial species of grasses, sedges, and broadleaf weeds. The relatively high water solubility and the ionic nature of glyphosate retard penetration through plant hydrophobic cuticular waxes. For this reason, glyphosate is commonly formulated with surfactants that decrease the surface tension of the solution and increase penetration into the tissues of plants (World Health Organization International Program on Chemical Safety, 1994; Giesy et al., 2000).

A large number of glyphosate-based formulations are registered in more than 100 countries and are available under different brand names. One of the most commonly applied glyphosate-based products is Roundup, containing glyphosate as the active ingredient (AI) and polyethoxylated tallowamine

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(POEA) as a surfactant. Glyphosate and its formulations have been extensively investigated for potential adverse effects in humans (Williams et al., 2000). This pesticide was reported to exert a low acute toxicity to different animal species. Experimental evidence showed that glyphosate did not bioaccumulate in any animal tissues (Williams et al., 2000). Chronic feeding studies in rodents did not find evidence of carcinogenic activity or any other relevant chronic effects (U.S. EPA, 1993; World Health Organization International Program on Chemical Safety, 1994).

With in vitro studies with tissue cultures or aquatic organisms, several of the formulated products are more toxic than glyphosate AI (Giesy et al., 2000; Williams et al., 2000). Differences in the response of test organisms to the AI and the commercial formulation, e.g., Roundup, are likely due to the toxicity of different formulants and surfactants contained in commercial products. There is a general agreement that adjuvants may be more toxic for animals than glyphosate itself (Giesy et al., 2000; Williams et al., 2000; Richard et al., 2005). Cytotoxicity of the commercial formulation Roundup to human peripheral mononuclear cells was 30-fold higher (LC_{50} = 56 mg/L) than for the AI (LC_{50} = 1640 mg/L) (Martinez et al., 2007). Several in vitro and in vivo studies with parallel testing of glyphosate AI and Roundup showed that only the commercial formulation was genotoxic (Rank et al., 1993; Bolognesi et al., 1997b; Gebel et al., 1997; Grisolia 2002). Cytotoxic and genotoxic effects were observed with Roundup and other formulations of glyphosate, but not with glyphosate AI alone in comparative studies involving different experimental systems (Peluso et al., 1998; Richard et al., 2005; Dimitrov et al., 2006). The observed differences were attributed to some ingredients of Roundup, mainly surfactants, and/or to a synergic effect of glyphosate and components of the formulation (Sirisattha et al., 2004; Peixoto 2005).

Epidemiological studies generally showed no consistent or strong relationships between human exposure to glyphosate or glyphosate-containing products and health outcomes in human populations. No statistically significant association in humans was found with spontaneous abortion, fetal deaths, preterm birth, neural tube defects (Rull et al., 2006), and cancer incidence overall, although a suggested association between cumulative exposure to glyphosate and the risk of multiple myeloma was reported (De Roos et al., 2005).The epidemiologic evidence is insufficient to verify a cause–effect relationship for childhood cancer (Wigle et al., 2008). Four case-control studies suggested an association between reported glyphosate use and the risk of non-Hodgkin's lymphoma (NHL) in age groups from 20 to 70 yr (Hardell & Eriksson, 1999; McDuffie et al., 2001; Hardell et al., 2002; De Roos et al., 2003; Eriksson et al., 2008).

Glyphosate AI and Roundup were extensively tested for genotoxicity in a wide range of in vitro and in vivo systems evaluating different genetic endpoints (gene mutation, chromosome mutation, DNA damage and repair) using bacteria and mammalian somatic cells (Williams et al., 2000). The active ingredient did not induce any relevant genotoxic effects such as gene mutations in a variety of in vitro bacterial assays including the *Salmonella typhimurium* reversion assay, with and without metabolic activation (Wildeman & Nazar 1982; Moriya et al., 1983; Li & Long, 1988) and *Escherichia coli* WP-2 (Moriya et al., 1983; Li & Long, 1988). The active ingredient was also negative in the Chinese hamster ovary cell HGPRT gene mutation assay and in primary hepatocyte DNA repair assay (Li & Long, 1988). The genotoxic potential of the formulation Roundup was investigated in a number of studies evaluating various genetic endpoints in different biological systems and was (1) negative in the *S. typhimurium* reversion assay (Kier et al., 1997), (2) negative in the sex-linked recessive lethal assay with *Drosophila melanogaster* (Gopalan & Njagi, 1981), and (3) negative for in vivo micronucleus (MN) induction in mouse bone marrow (Rank et al., 1993; Kier et al., 1997; Dimitrov et al., 2006). The Roundup formulation was reported in a number of studies to exert weak genotoxic effects in short-term assays.

Differences in the response of test organisms to the active ingredient glyphosate and the commercial formulation Roundup might be due to the toxicity of different co-formulants and surfactants contained in commercial products. Several studies with parallel testing of glyphosate and Roundup showed that only the commercial formulation was genotoxic (Rank et al., 1993; Bolognesi et al., 1997b; Gebel et al., 1997; Grisolia 2002). A recent study on the genotoxic potential of glyphosate formulations found that in some cases the genotoxic effects were obtained under exposure conditions that are not relevant for humans (Heydens et al., 2008).

An in vitro study described a concentration-dependent increase of DNA single-strand breaks (SSB), evaluated by comet assay, in two different human cell lines treated with glyphosate at sublethal concentrations (Monroy et al., 2005). Roundup formulations were shown to affect the cell cycle by inhibiting the G2/M transition and DNA synthesis leading to a genomic instability (Marc et al., 2004a, 2004b). Evidence of DNA damage in peripheral lymphocytes from a small group of subjects potentially exposed to glyphosate was reported in a recent paper (Paz-y-Miño et al., 2007). The number of subjects (21 control and 24 exposed) was small and there were 23 females and only 1 male in the exposed group, making interpretation of the results difficult.

Frequency of MN in human lymphocytes has been widely used for biomonitoring exposure to pesticides (Bolognesi, 2003; Costa et al., 2006; Montero et al., 2006). The MN test, an index of chromosomal damage, is one of the most appropriate biomarkers for monitoring a cumulative exposure to genotoxic agents. Chromosomal damage, as a result of inefficient or incorrect DNA repair, is expressed during the cell

division and represents an index of accumulated genotoxic effects. The cytokinesis-block micronucleus (CBMN) methodology (Fenech & Morley, 1985) allows a distinction to be made between a mononucleated cell that did not divide and a binucleated cell that has divided once, expressing any genomic damage associated to recent exposure. The test in its comprehensive application, as was proposed by Fenech (2007) including a set of markers of gene amplification, cellular necrosis, and apoptosis, allows evaluation of genotoxic and cytotoxic effects induced by exposure to a genotoxic agent.

Colombia's anti-drugs strategy includes a number of measures ranging from aerial spraying of a mixture of a commercial formulation of glyphosate (Glyphos) and an adjuvant, Cosmo-Flux (Solomon et al., 2007b), to manual eradication, including alternative development and crop substitution programs (UNODC, 2007). In order to assess the potential genotoxic risk associated with the aerial spraying program with the glyphosate mixture, a cytogenetic biomonitoring study was carried out in subjects from five Colombian regions, characterized by different exposure to glyphosate formulations and other pesticides.

MATERIALS AND METHODS

The study was carried out in five regions of Colombia, with different potential exposure to glyphosate as reported by Sanin et al. (2009). Briefly, the characteristics of the study areas are described here:

Sierra Nevada de Santa Marta—where organic coffee is grown without use of pesticides.

Boyacá—an area of illicit crops, where manual eradication is performed and the use of pesticides and other chemical agents is common.

Putumayo and Nariño—where aerial spraying of glyphosate is performed for coca and poppy eradication. The aerial application rate for eradication of coca is 3.69 kg glyphosate a.e. (acid equivalents)/ha (Solomon et al., 2007b). In order to maximize penetration and effectiveness of the spray formulation, Glyphos is tank-mixed with an adjuvant (Cosmo-Flux® 411F; Cosmoagro, Bogotá).

Valle del Cauca—where glyphosate is applied through aerial spraying for sugar cane maturation. Roundup 747 is the most commonly used product and is applied at a rate of 1 kg a.e./ha, and has no additional adjuvant (personal communication, ASOCAÑA, the Colombian Association for Sugar Growers, December 2008).

Study Population

Two hundred and seventy-four individuals were included in the study. The objective was to sample 30 couples of reproductive age in each area and, where possible, the same couples in the study conducted by Sanin et al. (2009) were sampled. In Putumayo, Nariño, and Valle del Cauca, the population was selected based on the scheduled aerial spraying of glyphosate. This schedule was confidential and provided exclusively for the purpose of the study by the Antinarcotics Police (Putumayo and Nariño) or ASOCAÑA (Valle del Cauca). In Valle del Cauca, a sample size of 30 couples could not be achieved because spraying was not carried out in populated areas of the study region. Most spraying during the study period was carried out on sugar cane crops where no inhabitants were found. All reported areas to be sprayed in Valle del Cauca were visited to search for couples; however, only 14 could be included.

In Sierra Nevada de Santa Marta and Boyacá, the same areas investigated in a previous study (Sanin et al., 2009) were identified, although, due to the instability of the population and high migration, most couples from the previous study were not located. In all regions, the same strategy as described before (Sanin et al., 2009) was followed, visiting household by household until completing 30 couples who fulfilled the inclusion criteria, women of reproductive age (15–49 yr of age) and their spouses, who voluntarily accepted to participate in the study.

Field Data Collection

Field data collection was carried out between October 2006 and December 2007. Epidemiologists and interviewers in the five regions who participated in the Sanin et al. (2009) study were informed about the objectives of the study and trained for data collection. The Ethical Committee of Fundacion Santa Fe de Bogotá approved the study protocol and the informed consent forms used for the study. All the subjects were informed about the aims of the study. All of them gave their informed consent and volunteered to donate blood for sampling. They did not self-report illness at the time of blood sampling and interviews. Every volunteer was interviewed with a standardized questionnaire, designed to obtain relevant details about the current health status, history, and lifestyle. This included information about possible confounding factors for chromosomal damage: smoking, use of medicinal products, severe infections or viral diseases during the last 6 mo, recent vaccinations, presence of known indoor/outdoor pollutants, exposure to diagnostic x-rays, and previous radio- or chemotherapy. A simplified food frequency questionnaire that had already been used in other regions of Colombia was also applied, in order to evaluate dietary folic acid intake. Folic acid intake was characterized because of the role of folic acid deficiency in baseline genetic damage in human lymphocytes (Fenech & Rinaldi, 1994). Specific information about exposure at the time of aerial spraying in Putumayo, Nariño, and Valle del Cauca was addressed in the questionnaire.

Blood Sampling and Cell Culture

Blood samples were collected twice in Boyacá, at the beginning of the study and 1 mo after the first survey, and at 3 different times in Nariño, Putumayo, and Valle del Cauca: immediately before spraying, within 5 d after spraying, and 4 mo later. A sample of 10 ml whole blood was collected from each subject, by venipuncture, using heparinized Vacutainer tubes kept at room temperature and sent within 24 h for the establishment of the lymphocyte cultures. The samples were coded before culturing. The modified cytokinesis-blocked method of Fenech and Morley (1985) was used to determine frequency of MN in lymphocytes. Whole blood cultures were set up for cytogenetic analysis in Bogotá (Colombia) by personnel specifically trained by cytogeneticists from Environmental Carcinogenesis Unit of the National Cancer Research Institute (Genoa, Italy).

Three sterile cultures of lymphocytes were prepared. A 0.4-ml aliquot of whole blood was incubated at 37°C in duplicate in 4.6 ml RPMI 1640 (Life Technologies, Milano, Italy) supplemented with 10% fetal bovine serum (Gibco BRL, Life Technologies SrL, Milano, Italy), 1.5% phytohemoagglutinin (Murex Biotech, Dartford, UK), 100 units/ml penicillin, and 100 µg/ml streptomycin. After 44 h, cytochalasin B (Sigma, Milano, Italy) was added at a concentration of 6 µg/ml. At the end of incubation at 37°C for 72 h, cells were centrifuged (800 × g, 10 min), then treated with 5 ml of 0.075 mM KCl for 3 min at room temperature to lyse erythrocytes. The samples were then treated with pre-fixative (methanol:acetic acid 3:1) and centrifuged . The cellular pellets were resuspended in 1 ml methanol. At this step the samples were sent to the Environmental Carcinogenesis Unit (National Cancer Research Institute, Genoa, Italy). All the samples were centrifuged in methanol. Treatment with fixative (methanol:acetic acid, 5:1) followed by centrifugation was repeated twice for 20 min. Lymphocytes in fresh fixative were dropped onto clean iced slides, air-dried, and stained in 2% Giemsa (Sigma, Milano, Italy). MN analysis was performed blind only on lymphocytes with preserved cytoplasm. On average, 2000 cells were analyzed for each subject. Cells were scored cytologically using the cytome approach to evaluate viability status (necrosis, apoptosis), mitotic status (mononucleated, binucleated, multinucleated) and chromosomal damage or instability status (presence of micronuclei, nucleoplasmic bridges, nucleoplasmic buds) (Fenech 2007). The proliferation index (PI) was calculated as follows:

$$PI = (\text{number of mononucleated cells} + 2 \times \text{number of binucleated cells} + 3 \times \text{number of polynucleated cells}) / \text{total number of cells}.$$

Statistical Analysis

Continuous variables were characterized using mean and standard deviation, while categorical variables were expressed as proportions. Dependent variables, micronuclei per binucleated cell (BNMN), and differences in MN between sampling were square-root transformed where required to comply with the required assumptions of normal distribution and equal variances. Comparison of MN between areas was made by one-way analysis of variance (ANOVA). A significance level at 5% was used to assess differences among areas. For multiple comparisons, the Bonferroni test was applied ($\alpha = .05$). Significance of differences in frequency of BNMN between first and second, and second and third sampling were tested by the unpaired t-test with equal variances. Difference and 95% confidence interval were used to compare between samplings.

Bivariate analysis between dependent variables and putative risk factors was performed by one-way ANOVA, comparing exposed and nonexposed subjects. In cases where risk factor was continuous, such as age, folic acid intake, alcohol consumption, and coffee consumption, the correlation coefficient was used.

A multiple linear regression was conducted to assess association with BNMN at the first sampling with different variables: region, age (as continuous variable as well as categorical age), ethnicity as a dichotomous variable, exposure to genotoxic products as defined earlier, gender (female vs. male), and intake of folic acid (categorized in quartiles). Regression analysis was conducted with transformed variables, with square root transformation of BNMN and natural logarithm of age, to obtain a normal distribution.

RESULTS

Demographic characteristics and habits of the study groups are described in Table 1. The study population comprised 274 subjects (137 female and 137 male; average age 30.4 ± 7.8 yr). The mean age of the subjects was similar in the different regions. A large part of the studied population was mestizo, with the exception of the Nariño area consisting of individuals of African origin. In the total population, 38% of interviewees had not completed primary education. Putumayo had the largest proportion with education and Valle del Cauca the lowest as shown in Table 1. Only 10% of all subjects were smokers, (20% in Putumayo); a large majority of subjects were drinkers of beer or liquor with a consistent consumption of guarapo (traditional alcoholic beverage prepared by fermentation of maize) in Santa Marta and Boyacá. No statistically significant differences of folic acid intake were observed between different regions (the mean values ranged from 750 and 1189 µg/wk).

One hundred and nine (39.8%) of 274 participants reported current use of pesticides in their occupation or other activities. Nariño (76.6%) and Putumayo (61.7%) were the two regions where prevalence of use of genotoxic pesticides was higher; Boyacá (24.2%) and Valle del Cauca (28.6%) reported lower use. None of the study subjects in Santa Marta reported use of pesticides. No data regarding quantity of pesticide used were available. Fifty (18.3%) out of 273 who gave information

TABLE 1
Demographic Characteristics and Possible Confounding Exposures in the Study Populations

Area	Santa Marta	Boyacá	Putumayo	Nariño	Valle del Cauca
Number of subjects	60	62	60	64	28
Age (mean (SD))	27.0 (5.6)	29.1 (8.8)	31.4 (7.2)	32.5 (7.4)	33.4 (8.7)
Ethnicity (%)					
Mestizo	100	100	88.3	3.1	60.7
African			6.7	96.9	39.3
Indian			5.0		
Education (%)					
None		4.8	1.7		
Primary incomplete	26.7	38.7	53.3	42.2	21.4
Primary complete	21.7	29.0	20.0	23.4	32.1
High school incomplete	25.0	8.1	20.0	25.0	28.6
High school complete	26.7	19.4	3.3	9.4	17.9
Technical			1.7		
Occupation (%)					
Agriculture	10.0	41.9	60.0	62.5	7.1
Housewife	40.0	50.0	38.3	34.4	50.0
Other	50.0	8.1	1.7	3.1	42.9
Health insurance (%)					
Uninsured	50.0	9.7	36.7	71.9	7.1
Subsidized	38.3	83.9	60.0	18.7	50.0
Insured	11.7	6.4	3.3	9.4	42.9
Coffee consumption (cups/day)					
Mean (SD)	1.8 (2.3)	1.7 (0.8)	2.3 (4.1)	1.3 (0.4)	1.7 (1.2)
Percent of population	80.0	67.7	88.3	76.6	82.1
Smoking (%)					
Nonsmokers	91.7	95.2	80.0	87.5	92.9
Alcohol (%)					
Liquor	28.3	25.8	53.3	78.1	78.6
Beer	51.6	67.7	63.1	82.8	64.3
Guarapo	6.7	59.7	1.7	3.2	10.7
Users of illicit drugs (%)	6.7	0	5.0	7.8	0
Diet					
Folic acid intake (µg/wk)	1189	873	750	1160	812

about x-ray examination reported to having been exposed at some time; however, only 21 out of 46 who gave information on dates of x-ray reported exposure in the last 6 mo before the interview and first blood sample. Sixty-one percent of population reported viral infections, the highest prevalence in Nariño (89.5%) and the lowest in Putumayo (49.2%). However, 89.3% of viral infections were the common cold and 6.1% dengue fever. Hepatitis was reported by six interviewees without any specification of the type of the infection.

The means and standard deviations of frequency of MN and related parameters according to regions are shown in Table 2 and presented graphically in Figure 1. Compared with Santa Marta, where people grow organic coffee without the use of pesticides and which is considered as a reference area, the baseline frequency of BNMN was significantly greater in subjects from the other four regions. The highest frequency of BNMN was in Boyacá, where no aerial eradication spraying of glyphosate was carried out, and Valle del Cauca, where aerial spraying was for maturation of sugar cane. There was no significant difference between mean frequency of BNMN in Boyacá and Valle del Cauca. There was no significant difference in frequency of BNMN between Putumayo and Nariño,

TABLE 2

Mean (SD) Frequency of Binucleated Cells with Micronuclei (BNMN), Total Micronuclei (MNL) per 1000 Binucleated Peripheral Lymphocytes, Frequency of Mononucleated Cells per 1000 Lymphocytes (MNMO), and Proliferation Index (PI) by Region before the Exposure (Phase 1), 5 d after Spraying (Phase 2) and 4 mo Later (Phase 3)

Region	Santa Marta	Boyacá	Putumayo	Nariño	Valle del Cauca
Phase 1					
Number of subjects	60	62	58	63	28
BNMN	1.83 (0.97)	5.64 (1.72)	3.61 (1.51)	4.12 (1.65)	5.75 (2.48)
MNL	1.97 (1.05)	6.16 (1.91)	3.90 (1.66)	4.36 (1.85)	6.02 (2.50)
MNMO	0.41 (0.44)	0.99 (0.64)	0.47 (0.51)	0.51 (0.39)	1.12 (0.88)
PI	1.54 (0.14)	1.45 (0.14)	1.68 (0.15)	1.47 (0.12)	1.51 (0.15)
Phase 2					
Number of subjects	ND	55	53	55	27
BNMN		4.96 (2.00)	4.64 (2.45)	5.98 (2.03)	8.64 (2.81)
MNL		5.41 (2.25)	5.02 (2.95)	6.35 (2.18)	8.98 (2.93)
MNMO		0.87 (0.65)	0.44 (0.46)	0.70 (0.45)	1.65 (0.62)
PI		1.72 (0.14)	1.66 (0.20)	1.40 (0.18)	1.51 (0.14)
Phase 3					
Number of subjects	ND	ND	50	56	26
BNMN			5.61(3.08)	3.91 (1.99)	7.38 (2.41)
MNL			5.96 (3.23)	4.13 (2.20)	8.17 (2.72)
MNMO			0.82 (0.54)	0.55 (0.42)	0.98 (0.60)
PI			1.43 (0.17)	1.41 (0.14)	1.45 (0.20)

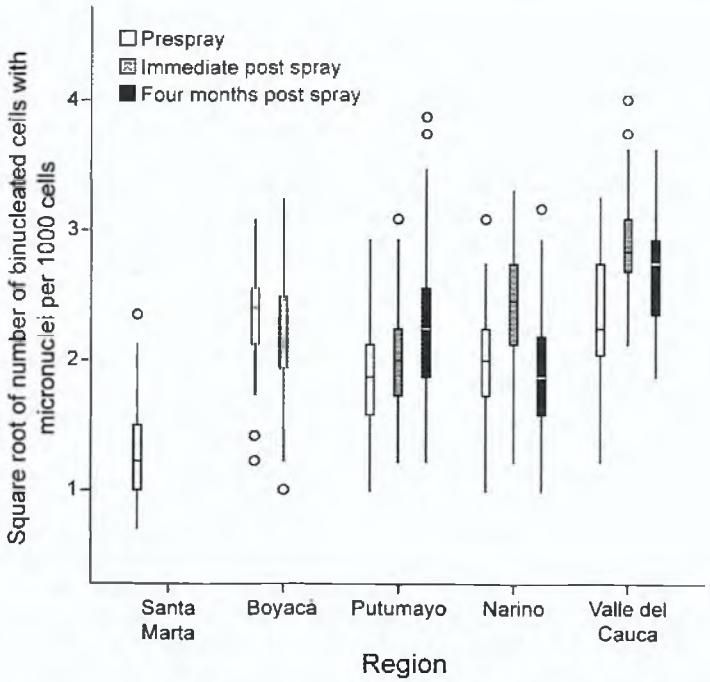


FIG. 1. Box plot of frequency of BNMN in the five study regions with samples taken prespray, 4–5 d post-spray, and 4 mo post-spray. Box plots: The center horizontal line marks the median of the sample. The length of each box shows the range within which the central 50% of the values fall, with the top and bottom of the box at the first and third quartiles. The vertical T-lines represent intervals in which 90% of the values fall. The ○ symbols show outliers. See text for description of statistically significant differences.

although Boyacá and Valle del Cauca showed a significantly higher frequency than Nariño and Putumayo. A higher frequency of BNMN in Boyacá was also observed in a second sampling 1 mo later.

There were differences in frequency of BNMN between sampling periods. A statistically significant difference in frequency of BNMN between first and second sampling was observed in Valle, Putumayo, and Nariño immediately (<5 d) after spraying. Four months after spraying in Nariño, there was a statistically significant decrease in the mean frequency of BNMN compared with the second sampling, but in Valle del Cauca the decrease was not significant nor was the increase observed in Putumayo significant (Figure 1 and Table 2).

The frequency of mononucleated cells with micronuclei (MOMN) was used as an index of background level of chromosomal damage accumulated in vivo (Table 2). The lowest frequency of MOMN for the first sampling was observed in Santa Marta; however, there was no marked difference in frequency of MOMN in Santa Marta, Putumayo, and Nariño and no statistically significant difference between Valle and Boyacá. However, Valle and Boyacá had a significantly higher frequency of MOMN than Putumayo, Nariño, and Santa Marta at first sampling. Immediately after spraying, Valle showed a significantly higher frequency of MOMN compared to Putumayo and Nariño, and Nariño was also higher than Putumayo. Between first and second sampling, the increase in frequency of MOMN in Nariño and Valle was statistically significant, but there was no difference in Putumayo nor in Boyacá 4 mo after the first sampling. Data suggest greater exposure to genotoxic agents in these populations is independent of the exposure to glyphosate products.

The proliferation index (PI) in all the studied groups was in the range of normal values described in the literature. No significant reduction of PI was observed in association with environmental exposures in groups of subjects from the different regions. A statistically significant correlation coefficient (0.288) between PI values from the first and the second samplings was observed, confirming the association with individual characteristics and not with any toxicity related to the exposure or to the culture techniques. Due to the low frequency observed, data with respect to other nuclear alterations, including in cytome analysis (Fenech, 2007), are not described in Table 2: the mean frequency of nucleoplasmic bridges (NPB) for all subjects was 0.010 per 1000 cells, that of nuclear buds was 0.022 per 1000 cells, and only rare necrotic and apoptotic cells were found in some samples.

Gender was the most important demographic variable affecting the BNMN index. Frequencies of BNMN in females were greater than those in males (mean 4.43 ± 2.36 vs. 3.61 ± 1.82, respectively, in total population) (Table 3). The groups of subjects were evenly matched for gender by including only couples in the study. No association was found between frequency of MN and age as a categorical variable, nor was there an association with smoking, but prevalence of smoking was low (~10% in the total population). A higher baseline frequency of MN was observed in subjects of African origin, suggesting greater susceptibility. Other lifestyle factors such as alcohol, coffee consumption, or illicit drug intake were not associated with initial measures of BNMN and MOMN.

One hundred and thirty-four of the 152 subjects in Nariño, Putumayo, and Valle reported information on contact with Glyphos and Cosmo-Flux after eradication spraying. The other 18 did not provide information in the second survey or blood samples were inadequate for testing micronuclei. Sixty-six (49.2.0%) reported no contact with the spray and 68 (50.8%) reported coming into contact with the spray because they entered sprayed fields or reported contact with the spray droplets. The mean BNMN in Nariño and Putumayo was greater in respondents who self-reported exposure, but differences were not statistically significant (Table 4). In Valle, only one respondent reported contact with glyphosate.

Region, gender, and older age (≥35 yr) were the only variables associated with the frequency of BNMN before spraying (Table 5). In fact, using Santa Martha, where no use of pesticides was reported, as reference, Boyacá, Valle del Cauca, Putumayo, and Nariño showed a statistically significant higher mean frequency of BNMN. There were also significant differences between Boyacá and Valle and Putumayo and Nariño. Females had a statistically higher mean frequency of BNMN than males after adjusting for all other variables. Greater age was also associated with greater frequency of BNMN. Neither exposure to genotoxic products, nor ethnicity, nor intake of folic acid was associated with frequency of BMMN at the first sampling. The multiple linear regression analysis of difference between second and first sampling only demonstrated statistically significant association with region after adjusting for all other variables, indicating that Putumayo, Nariño, and Valle had significantly greater differences between second and first sampling than Boyacá.

DISCUSSION

The main objective of this study was to test whether there was an association between aerial spraying of glyphosate and cytogenetic alterations, evaluated as frequency of MN in peripheral leukocytes. Biomonitoring was carried out in three regions of Colombia in populations exposed to aerial spraying of glyphosate: Putumayo and Nariño, where the application was performed for eradication of coca and poppy, and Valle del Cauca where the herbicide was used for maturation of sugar cane. Two control populations not exposed to aerial spraying of glyphosate were also selected: the first one from Sierra Nevada de Santa Marta, where organic coffee is grown without the use of any pesticides, and the other from Boyacá, with a region of illicit crops, where manual eradication is performed and subjects were potentially exposed to several pesticides but not glyphosate for aerial eradication. The ex vivo analysis of leukocytes in the presence of cytochalasin B, added 44 h after the

TABLE 3
Association of Mean (SD) Frequency of Binucleated Cells (First Sampling) with Micronuclei (BNMN/1000 Binucleated Lymphocytes) and Demographic Variables

Variable	Santa Marta	Boyacá	Putumayo	Nariño	Valle del Cauca	Total
Sex						
Females	1.98 (1.03)	6.22 (1.79)	3.91 (1.71)	4.57(1.77)	6.45 (2.82)	4.43 (2.36)
Males	1.68 (0.90)	5.06 (1.46)	3.31 (1.25)	3.66 (1.39)	5.05 (1.94)	3.61 (1.82)
p	.236	.007	.131	.028	.138	.002
Age						
18–24 yr	2.00 (1.14)	5.50 (1.96)	3.32 (1.25)	3.64 (1.72)	6.19 (2.15)	3.67 (2.16)
25–34 yr	1.66 (0.87)	5.70 (1.66)	3.53 (1.17)	4.20 (1.77)	4.20 (0.76)	3.97 (2.08)
35 yr and older	1.93 (0.67)	5.62 (1.73)	3.84 (1.86)	4.25 (1.52)	6.04 (2.84)	4.41 (2.19)
p	.438	.929	.574	.564	.313	.093
Ethnicity						
Mestizo	1.83 (0.97)	5.64 (1.72)	3.72 (1.52)	4.75 (1.06)	5.82 (2.44)	3.94(2.24)
Africa and Indian	0	0	2.86 (1.31)	4.10 (1.66)	5.64 (2.65)	4.20(1.90)
p			.162	.588	.850	.368
Smoking						
Yes	2.00 (1.06)	5.33 (0.76)	3.31 (1.00)	4.77 (1.51)	4.50 (1.41)	3.83 (1.60)
No	1.82 (0.97)	5.65 (1.76)	3.80 (1.56)	4.03 (1.66)	5.90 (2.57)	4.07 (2.20)
p	.693	.756	.395	.233	.459	.592
Folic acid intake (quartiles)						
1	1.92 (0.99)	6.11 (1.95)	3.23 (1.12)	4.50 (1.75)	5.86 (2.34)	3.89 (2.23)
2	1.64 (0.66)	5.70 (1.75)	3.47 (1.49)	3.80 (1.47)	5.86 (2.74)	3.97 (2.21)
3	1.69 (0.92)	5.69 (1.82)	4.00 (1.37)	3.85 (2.04)	6.58 (2.84)	4.47 (2.22)
4	1.94 (1.20)	4.94 (1.13)	3.69 (2.429)	4.28 (1.51)	4.63 (2.05)	3.75 (1.89)
p	.779	.399	.515	.645	.612	.220

TABLE 4
Mean Frequency of Binucleated Cells with Micronuclei (BNMN) at the Second Sampling per 1000 Binucleated Lymphocytes and Self-Reported Exposures to the Glyphosate Spray in Three Areas Where Aerial Application Had Occurred

	Nariño (*n* = 55)		Putumayo (*n* = 53)		Valle del Cauca (*n* = 26)	
Route of exposure	*n*	Mean BNMN (SD)	*n*	Mean BNMN (SD)	*n*	Mean BNMN (SD)
No exposure	28	5.81 (1.85)	13	3.84 (1.30)	25	8.56 (2.90)
Spray in air	5	7.30 (0.57)	1	5.50 (0)		
Spray on skin	8	5.62 (1.60)	15	4.90 (1.87)	1	9.50 (0)
Entered sprayed field	14	6.06 (2.77)	24	4.87 (3.18)		
p Value (ANOVA)		0.472		0.612		0.760
Any exposure	27	6.16 (2.22)	40	4.90 (2.69)	1	9.50 (0)
p Value (no exposure vs. any exposure)		0.525		0.181		0.760

Note. The data comprise respondents in the second survey from which blood samples were obtained.

TABLE 5
Multiple Linear Regression Analysis Adjusted for Region, Age, Gender, Ethnicity, and Folic Acid Intake

Variable	Coefficient	*p*	95% CI
Region			
Boyacá	3.75	≤.0001	3.19, 4.31
Putumayo	1.58	≤.0001	1.00, 2.16
Nariño	2.06	≤.0001	1.49, 2.64
Valle del Cauca	3.65	≤.0001	2.92, 4.39
Age (yr)			
25–34	0.28	.250	–0.20, 0.76
35 and older	0.75	.008	0.20, 1.31
Gender			
Females	1.00	≤.0001	0.60, 1.40

start of cultivation, made it possible to distinguish between non-dividing mononucleated cells—as an index of accumulated chromosomal damage—and binucleated cells, which had completed one nuclear division during in vitro culture and expressed MN associated with recent exposure to genotoxic agents.

The baseline level of chromosomal damage, evaluated as frequency of BNMN, was associated with the different regions considered in our study. The frequency of BNMN before spraying was also associated with region, gender, and age. Gender difference in the background incidence of MN in peripheral leukocytes, with the frequency being consistently higher in females, and a strong correlation between MN frequency and increasing age are well documented (Bonassi et al., 1995, 2001; Bolognesi et al., 1997a).

Data demonstrated no significant effect of smoking, confirming findings from the literature (Bonassi et al., 2003) although prevalence of smoking in our study population was small (7–20%, Table 1). No association with alcohol consumption was observed. A higher susceptibility of people of African origin compared to the mestizo group was suggested by a greater baseline frequency of BNMN and increased frequency at the second sampling period.

There was some indication of an association between BNMN and exposure to pesticides in general. The lowest frequency of BNMN was observed in Sierra Nevada de Santa Marta, where people self-reported that they did not use pesticides. The mean frequency of BNMN in this group of subjects (1.83 ± 0.97) was similar to that observed in healthy unexposed subjects for the same range of age (Bolognesi et al., personal communication). The higher mean frequency of BNMN observed in Boyacá and Valle del Cauca (5.64 ± 1.72 and 5.75 ± 2.48, respectively) and that in Nariño and Putumayo (4.12 ± 1.65 and 3.65 ± 1.51, respectively), compared to Santa Marta, are in agreement with similar biomonitoring studies carried out in subjects exposed to pesticides using the MN test or other genetic endpoints (Bolognesi, 2003; Bull et al., 2006).

There was no clear relationship between BNMN and the reported use of pesticides classified as genotoxic. Participants in Boyacá and Valle del Cauca showed higher frequency of BNMN than those in Putumayo and Nariño. However, a greater proportion of participants in the latter regions self-reported the use genotoxic pesticides (76.6% in Nariño and 61.7% in Putumayo). There is no information available on other relevant factors such as frequency of use, rate applied, time of exposure, and protective measures used, and we could therefore not characterize exposures to explain the differences. There were further inconsistencies; for example, in Boyacá, where more frequent use of pesticides was expected, only 24.2% of participants self-reported use, compared with the greater values in Nariño and Putumayo. However, it is possible that in areas such as Boyacá, individuals might be potentially exposed to persistent pesticides applied in the past and still present in the environment.

There was no evidence of an association between BNMN and folic acid deficiency. An assessment of folic acid intake from the semiquantitative food frequency questionnaire showed that, according to accepted recommendations (Herbert, 1987), the diet of the study populations was not deficient in folic acid and there were only small differences between regions. Consistent with these data, no association was found between MN and folic acid intake, either as a continuous variable or by quartiles.

The frequency of BNMN increased after spraying with glyphosate but not consistently. The results obtained with a second sampling, carried out immediately after the glyphosate spraying, showed a statistically significant increase in frequency of BNMN in the three regions where glyphosate was sprayed. However, this was not consistent with the rates of application use in the regions. The increase in frequency of BNMN in Valle (application rate = 1 kg a.e. glyphosate/ha) was greater than that in Nariño and Putumayo (3.69 kg a.e. glyphosate/ha).

There was no significant association between self-reported direct contact with eradication sprays and frequency of BNMN. The frequency of BNMN in participants who self-reported that they were exposed to glyphosate because they entered the field immediately after spraying (to pick the coca leaves), felt spray drops in their skin, or they thought they were exposed because they had contact with the chemical in the air, was not significantly greater than in subjects living in the same areas but who were not present during spraying. Decreases in frequency of BNMN in the recovery period after glyphosate spraying were not consistent. The third sampling, 4 mo after spraying, demonstrated a statistically significant decrease in frequency of BNMN only in Nariño.

Overall, these results suggest that genotoxic damage associated with glyphosate spraying, as evidenced by the MN test, is small and appears to be transient. The frequencies of BNMN in Nariño and Putumayo during the second and the third sampling fell within the range of values observed in Boyacá, an area

where people were exposed to a complex mixture of different pesticides (including glyphosate). A greater increase in frequency of BNMN was observed in Valle del Cauca, but it cannot be attributed only to the glyphosate exposure, because the application rate of the herbicide in this area was one-third compared with that in Nariño and Putumayo. This conclusion is further supported by the frequency of MN in mononucleated cells (MOMN), which provides an indication of the background level of chromosome/genome mutations accumulated in vivo (Manteuca et al., 2006). A statistically significant increase of MOMN was observed in Boyacá and Valle del Cauca before and after the aerial spraying, suggesting exposure to other genotoxic compounds in these populations was independent of the exposure to glyphosate. Evidence indicates that the genotoxic risk potentially associated with exposure to glyphosate in the areas where the herbicide is applied for eradication of coca and poppy is of low biological relevance. One of the strengths of our study was the detection of a transient chromosomal damage, evaluated as MN frequency in peripheral blood of the exposed subjects, since it was possible to compare the baseline before spraying with the effects detected immediately after spraying. Glyphosate persists in the environment for only a short time (half-life for biological availability in soil and sediments is hours, and 1-3 d in water; Giesy et al., 2000), is rapidly excreted by mammals and other vertebrates (Williams et al., 2000; Acquavella et al., 2004) and chronic effects, if any, would not be expected.

One of the major drawbacks of environmental epidemiology studies is the characterization of exposures to the agents being investigated. In this study two approaches were used to characterize exposures to glyphosate: ecological and self-reported. In the ecological study design, frequency of BNMN in participants was compared from regions with different patterns of pesticide use. As previously discussed (Sanín et al., 2009), this ecological design may result in misclassification of exposures (Arbuckle et al., 2004), but as an exploratory assessment of exposure it is useful (Ritter et al., 2006).

Others have attempted to improve assessment of exposure to pesticides in epidemiological studies. One study used a self-administered questionnaire for the assessment of exposure to glyphosate, which was defined as (a) ever personally mixed or applied products containing glyphosate; (b) cumulative lifetime days of use, or "cumulative exposure days" (years of use times days/year); and (c) intensity-weighted cumulative exposure days (years of use times days/year times estimated intensity level) (De Roos et al., 2005). A pesticide exposure score based on self-reported work practices was recently developed to estimate annual exposure level (Firth et al., 2007). Based on an algorithm to estimate lifetime exposure to glyphosate from questionnaire information, a moderate correlation was found with concentrations of glyphosate in urine and no significant correlation with self-reported exposure (Acquavella et al., 2004).

In our study, questions related to whether there was direct contact with the spray were used but this did not consider area of skin exposed, region of skin exposed, differences in rates of penetration, or personal hygiene.

Given the situation, the best approach possible, a prospective cohort, was used but the need to use better procedures to estimate the exposure is acknowledged. Based on the applicable Bradford–Hill guidelines (Hill, 1965), it is not possible to assign causality to the increases in frequency of BNMN observed in our study. There was a smaller frequency of BNMN and MOMN in the region of no pesticide use compared with the regions where pesticides (including glyphosate) were used, which is consistent with other reports in the literature. Although temporality was satisfied in the increase in frequency of BNMN after spraying, this response did not show strength as it was not consistently correlated with the rate of application. Recovery was also inconsistent with decreases in frequency of BNMN in the areas of eradication spraying but not in the area where lower rates were applied on sugar cane.

Further studies are needed to better characterize the potential genotoxic risk associated with the application of glyphosate for sugar cane maturation. The smaller number of subjects recruited in this study and small amount of information about the exposure precluded any conclusions. Many pesticides are used in conventional agriculture in Colombia and many pesticides are used in the production of coca (Solomon et al., 2007a, 2007b); however, there is not sufficient information to correlate the frequency of MN to the pesticide exposure.

REFERENCES

Acquavella, J. F., Alexander, B. H., Mandel, J. S., Gustin, C., Baker, B., Chapman, P., and Bleeke, M. 2004. Glyphosate biomonitoring for farmers and their families: Results from the farm family exposure study. *Environ. Health Perspect.* 112:321–326.

Arbuckle, T. E., Cole, D. C., Ritter, L., and Ripley, B. D. 2004. Farm children's exposure to herbicides: Comparison of biomonitoring and questionnaire data. *Epidemiology* 15:187–194.

Baylis, A. D. 2000. Why glyphosate is a global herbicide: Strengths, weaknesses and prospects. *Pestic. Manage. Sci.* 56:299–308.

Bolognesi, C. 2003. Genotoxicity of pesticides: A review of human biomonitoring studies. *Mutat. Res.* 543:251–272.

Bolognesi, C., Abbondandolo, A., Barale, R., Casalone, R., Dalpraà, L., De Ferrari, M., Degrassi, F., Forni, A., Lamberti, L., Lando, C., Migliore, L., Padovani, D., Pasquini, P., Puntoni, R., Sbrana, I., Stella, M., and Bonass, S. 1997a. Age-related increase of baseline frequencies of sister chromatid exchanges, chromosome aberrations, and micronuclei in human lymphocytes. *Cancer Epidemiol. Biomarkers. Prev.* 6:249–256.

Bolognesi, C., Bonatti, S., Degan, P., Gallerani, E., Peluso, M., Rabboni, R., Roggieri, P., and Abbondandolo, A. 1997b. Genotoxic activity of glyphosate and its technical formulation, Roundup. *J. Agric. Food. Chem.* 45:1957–1962.

Bonassi, S., Bolognesi, C., Abbondandolo, A., Barale, R., Bigatti, P., Camurri, L., Dalpra, L., De Ferrari, M., Forni, A., Lando, C., Padovani, P., Pasquini, R., Stella, M., and Puntoni, R. 1995. Influence of sex on cytogenetic endpoints: Evidence from a large human sample and review of the literature. *Cancer Epidemiol. Biomarkers. Prev.* 4:671–679.

Bonassi, S., Fenech, M., Lando, C., Lin, Y. P., Ceppi, M., Chang, W. P., Holland, N., Kirsch-Volders, M., Zeiger, E., Ban, S., Barale, R., Bigatti, M., Bolognesi, C., Jia, C., Di Giorgio, M., Ferguson, L. R., Fucic, A., Lima, O. G., Hrelia, P., Krishnaja, A. P., Lee, T. K., Migliore, L., Mikhalevich, L.,

Mirkova, E., Mosesso, P., Müller, W. U., Odagiri, Y., Scarffi, M. R., Szabova, E., Vorobtsova, I., Vral, A., and Zijno, A. 2001. HUman Micro-Nucleus project: International database comparison for results with the cytokinesis-block micronucleus assay in human lymphocytes: I. Effect of laboratory protocol, scoring criteria, and host factors on the frequency of micronuclei. *Environ. Mol. Mutagen.* 37:31–45.

Bonassi, S., Neri, M., Lando, C., Ceppi, M., Lin, Y.-P., Chang, W. P., Holland, N., Kirsch-Volders, M., Zeiger, E., Fenech, M., and The HUMN collaborative group. 2003. Effect of smoking habit on the frequency of micronuclei in human lymphocytes: Results from the Human MicroNucleus project. *Mutat. Res.* 543:155–166.

Bull, S., Fletcher, K., Boobis, A. R., and Battershill, J. M. 2006. Evidence for genotoxicity of pesticides in pesticide applicators: A review. *Mutagenesis* 21:93–103.

Costa, C., Teixeira, J. P., Silva, S., Roma-Torres, J., Coelho, P., Gaspar, J., Alves, M., Laffon, B., Rueff, J., and Mayan, O. 2006. Cytogenetic and molecular biomonitoring of a Portuguese population exposed to pesticides. *Mutagenesis* 21:343–350.

De Roos, A. J., Blair, A., Rusiecki, J. A., Hoppin, J. A., Svec, M., Dosemeci, M., Sandler, D. P., and Alavanja, M. C. 2005. Cancer incidence among glyphosate-exposed pesticide applicators in the Agricultural Health Study. *Environ. Health Perspect.* 113:49–54.

De Roos, A. J., Zahm, S. H., Cantor, K. P., Weisenburger, D. D., Holmes, F. F., Burmeister, L. F., and Blair, A. 2003. Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. *Occup. Environ. Med.* 60:E11.

Dimitrov, B. D., Gadeva, P. G., Benova, D. K., and Bineva, M. V. 2006. Comparative genotoxicity of the herbicides Roundup, Stomp and Reglone in plant and mammalian test systems. *Mutagenesis* 21:375–382.

Duke, S. O., and Powles, S. B. 2008. Glyphosate: A once-in-a-century herbicide. *Pestic. Manage. Sci.* 64:319–325.

Eriksson, M., Hardell, L., Carlberg, M., and Akerman, M. 2008. Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis. *Int. J. Cancer* 123:1657–1663.

Fenech, M. 2007. Cytokinesis-block micronucleus cytome assay. *Nat. Prot.* 2:1084–1104.

Fenech, M., and Morley, A. A. 1985. Measurement of micronuclei in lymphocytes. *Mutat. Res.*, 147:29–36.

Fenech, M., and Rinaldi, J. 1994. The relationship between micronuclei in human lymphocytes and plasma levels of vitamin C, vitamin E, vitamin B12 and folic acid. *Carcinogenesis* 15:1405–1411.

Firth, H. M., Rothstein, D. S., Herbison, G. P., and McBride, D. I. 2007. Chemical exposure among NZ farmers. *Int. J. Environ. Health. Res.* 17:33–44.

Gebel, T., Kevekordes, S., Pav, K., Edenharder, R., and Dunkelberg, H. 1997. In vivo genotoxicity of selected herbicides in the mouse bone marrow micronucleus test. *Arch. Toxicol.* 71:193–197.

Giesy, J. P., Dobson, S., and Solomon, K. R. 2000. Ecotoxicological risk assessment for Roundup® herbicide. *Rev. Environ. Contam. Toxicol.* 167:35–120.

Gopalan, H. N. B., and Njagi, G. D. E. 1981. Mutagenicity testing of pesticides: III. *Drosophila*: Recessive sex-linked lethals. *Genetics* 97(Suppl.):S44.

Grisolia, C. K. 2002. A comparison between mouse and fish micronucleus test using cyclophosphamide, mitomycin C and various pesticides. *Mutat. Res.* 518:145–150.

Hardell, L., and Eriksson, M. 1999. A case-control study of non-Hodgkin lymphoma and exposure to pesticides. *Cancer* 85:1353–1360.

Hardell, L., Eriksson, M., and Nordstrom, M. 2002. Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: Pooled analysis of two Swedish case-control studies. *Leuk. Lymphoma* 43:1043–1049.

Herbert, V. 1987. Recommended dietary intakes (RDI) of folate in humans. *Am. J. Clin. Nutr.* 45:661–670.

Heydens, W. F., Healy, C. E., Hotz, K. J., Kier, L. D., Martens, M. A., Wilson, A. G. E., and Farmer, D. R. 2008. Genotoxic potential of glyphosate formulations: Mode-of-action investigations. *J. Agric. Food. Chem.* 56:1517–1523.

Hill, A. B. 1965. The environment and disease: association or causation? *Proc. R. Soc. Med.* 58:295–300.

Kier, L. D., Stegeman, S. D., Dudek, S., McAdams, J. G., Flowers, F. J., Huffman, M. B., and Heydens, W. F. 1997. Genotoxicity studies of glyphosate, alachlor and butachlor formulations. *Fundam. Appl. Toxicol.* 36:305.

Li, A. P., and Long, T. J. 1988. An evaluation of genotoxic potential of glyphosate. *Fundam. Appl. Toxicol.* 10:537–546.

Manteuca, R., Lombaert, N., V, A. P., Decordier, I., and Kirsch-Volders, M. 2006. Chromosomal changes: induction, detection methods and applicability in human biomonitoring. *Biochimie* 88:1515–1531.

Marc, J., Bellé, R., Morales, J., Cormier, P., and Mulner-Lorillon, O. 2004a. Formulated glyphosate activates the DNA-response checkpoint of the cell cycle leading to the prevention of G2/M transition. *Toxicol. Sci.* 82:436–442.

Marc, J., Mulner-Lorillon, O., and Bellé, R. 2004b. Glyphosate-based pesticides affect cell cycle regulation. *Biol. Cell.* 96:245–247.

Martinez, A., Reyes, I., and Reyes, N. 2007. Cytotoxicity of the herbicide glyphosate in human peripheral blood mononuclear cells. *Biomédica* 27:594–604.

McDuffie, H. H., Pahwa, P., McLaughlin, J. R., Spinelli, J. J., Fincham, S., Dosman, J. A., Robson, D., Skinnider, L., and Choi, N. W. 2001. Non-Hodgkin's lymphoma and specific pesticide exposures in men: Cross-Canada study of pesticides and health. *Cancer Epidemiol. Biomarkers. Prev.* 10:1155–1163.

Monroy, C. M., Cortes, A. C., Sicard, D. M., and Groot, H. 2005. Citotoxicidad y genotoxicidad de células humanas espuestas in vitro a glifosato. *Biomédica* 25:335–345.

Montero, R., Serrano, L., Araujo, A., Davila, V., Ponce, J., Camacho, R., Morales, E., and Mendez, A. 2006. Increased cytogenetic damage in a zone in transition from agricultural to industrial use: Comprehensive analysis of the micronucleus test in peripheral blood lymphocytes. *Mutagenesis* 21:335–342.

Moriya, M., Ohta, T., Watanabe, K., Miyasawa, T., Kato, K., and Shirasu, Y. 1983. Further mutagenicity studies on pesticides in bacterial reversion assay systems. *Mutat. Res.*, 116:185–216.

Paz-y-Miño, C., Sánchez, M. E., Arévalo, M., Muñoz, M. J., Witte, T., De-la-Carrera, G. O., and Paola, L. E. 2007. Evaluation of DNA damage in an Ecuadorian population exposed to glyphosate. *Genet. Mol. Biol.* 30:456–460.

Peixoto, F. 2005. Comparative effects of the Roundup and glyphosate on mitochondrial oxidative phosphorylation. *Chemosphere* 61:1115–1122.

Peluso, M., Munnia, A., Bolognesi, C., and Parodi, S. 1998. ^{32}P-postlabeling detection of DNA adducts in mice treated with the herbicide Roundup. *Environ. Mol. Mutagen.* 31:55–59.

Rank, J., Jensen, A. G., Skov, B., Pedersen, L. H., and Jensen, K. 1993. Genotoxicity testing of Roundup and its active ingredient glyphosate isopropylamine using the mouse bone marrow micronucleus test, *Salmonella* mutagenicity test and *Allium* anaphase-telophase test. *Mutat. Res.* 300:29–36.

Richard, S., Moslemi, S., Sipahutar, H., Benachour, N., and Seralini, G.-E. 2005. Differential effects of glyphosate and Roundup on human placental cells and aromatase. *Environ. Health Perpect.* 113:716–720.

Ritter, L., Goushleff, N. C. I., Arbuckle, T., Cole, D., and Raizenne, M. 2006. Addressing the linkage between exposure to pesticides and human health effects—Research trends and priorities for research 1. *J. Toxicol. Environ. Health B* 9:441–456.

Rull, R. P., Ritz, B., and Shaw, G. M. 2006. Neural tube defects and maternal residential proximity to agricultural pesticide applications. *Am. J. Epidemiol.* 163:743–753.

Sanin, L.-H., Carrasquilla, G., Solomon, K. R., Cole, D. C., and Marshall, E. J. P. 2009. Regional differences in time to pregnancy among fertile women from five Colombian regions with different uses of glyphosate. *J. Toxicol. Environ. Health A* 72:949–960.

Sirisattha, S., Momse, Y., Kitagawa, E., and Iwahashi, H. 2004. Genomic profile of roundup treatment of yeast using DNA microarray analysis. *Environ. Sci.* 11:313–323.

Solomon, K. R., Anadón, A., Brain, R. A., Cerdeira, A. L., Crossan, A. N., Marshall, A. J., Sanin, L. H., and Smith, L. 2007a. Comparative hazard assessment of the substances used for production and control of coca and poppy in Colombia. In *Rational environmental management of agrochemicals: Risk assessment, monitoring, and remedial action. ACS Symposium Series no. 966* (vol. 966), eds. Kennedy, I. R., Solomon, K. R., Gee, S., Crossan, A. N., Wang, S., and Sanchez-Bayo, F. pp. 87–99. Washington, DC: American Chemical Society.

Solomon, K. R., Anadón, A., Carrasquilla, G., Cerdeira, A., Marshall, J., and Sanin, L.-H. 2007b. Coca and poppy eradication in Colombia: Environmental and human health assessment of aerially applied glyphosate. *Rev. Environ. Contam. Toxicol.* 190:43–125.

UNODC. 2007. *World drug report 2007*. United Nations Office on Drugs and Crime. Accessed January 29, 2008. http://www.unodc.org

U.S. Environmental Protection Agency. 1993. *R.E.D. Facts Glyphosate*. Technical report EPA 738-R-93-014. Washington, DC: U.S. Environmental Protection Agency.

Wigle, D. T., Arbuckle, T. E., Turner, M. C., Berube, A., Yang, Q., Lui, S., and Krewski, D. 2008. Epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants. *J. Toxicol. Environ. Health B* 11:373–517.

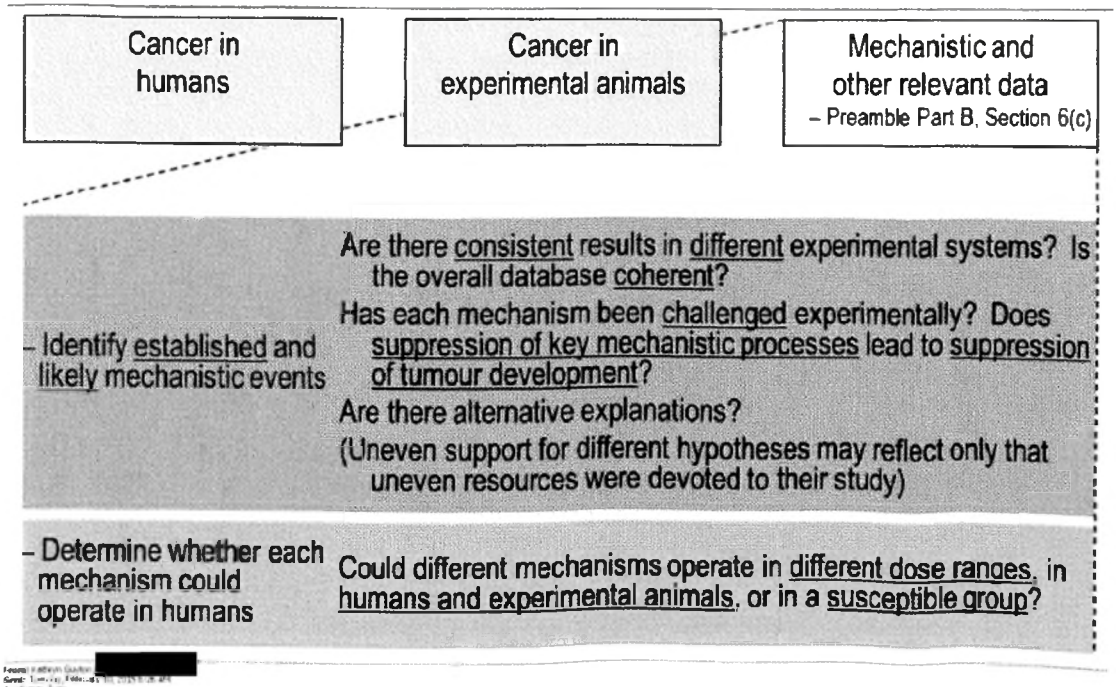
Wildeman, A. G., and Nazar, R. N. 1982. Significance of plant metabolism in the mutagenicity and toxicity of pesticides. *Can. J. Genet. Cytol.* 24:437–449.

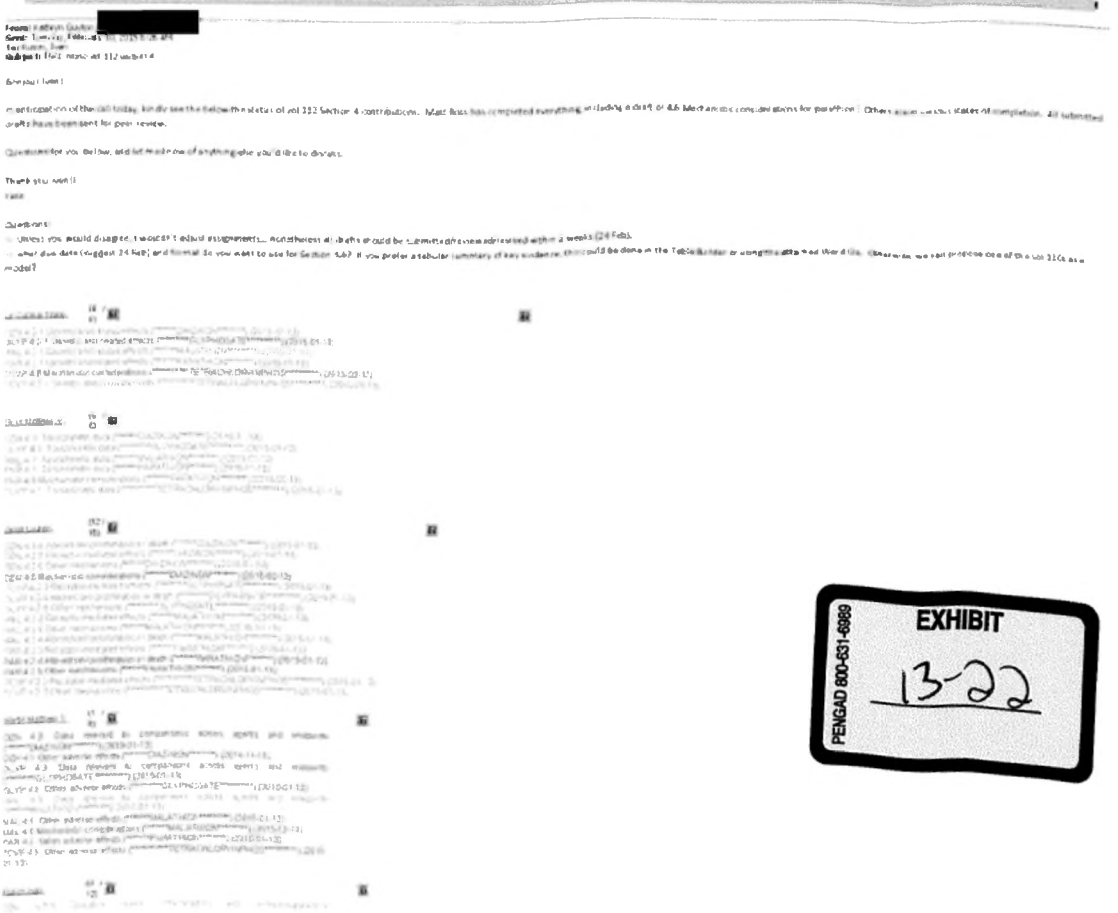
Williams, G. M., Kroes, R., and Munro, I. C. 2000. Safety evaluation and risk assessment of the herbicide Roundup® and its active ingredient, glyphosate, for humans. *Regul. Toxicol. Pharmacol.* 31:117–165.

Woodburn, A. T. 2000. Glyphosate: production, pricing and use worldwide. *Pestic. Manage. Sci.* 56:309–312.

World Health Organization International Program on Chemical Safety. 1994. *Glyphosate* (vol. 159). Geneva: WHO IPCS.

002915


Cancer in humans
Cancer in experimental animals
Mechanistic and other relevant data – Preamble Part B, Section 6(c)
– Identify established and likely mechanistic events
Are there consistent results in different experimental systems? Is the overall database coherent?
Has each mechanism been challenged experimentally? Does suppression of key mechanistic processes lead to suppression of tumour development?
Are there alternative explanations?
(Uneven support for different hypotheses may reflect only that uneven resources were devoted to their study)
– Determine whether each mechanism could operate in humans
Could different mechanisms operate in different dose ranges, in humans and experimental animals, or in a susceptible group?



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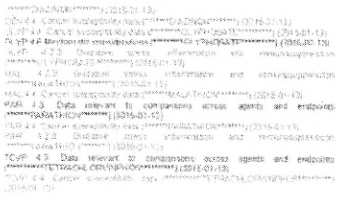

005429

From: Kathryn Guyton
To: LE CURIEUX Frank
Cc: Andy Shapiro; Ross, Matthew; Matt Martin; Lauren Zeise; Rusyn, Ivan
Subject: Re: Thanks!
Date: Friday, March 13, 2015 9:18:56 AM

Dear Frank,

A great suggestion. Unfortunately I, among other toxicologists, don't understand the epidemiologists and their exposure compadres. However, I agree that their input (whatever it meant) on the Bolognesi study was critical and, in the end, as valuable as "sheep dip". ;-).

Please enjoy the attached photo; as they say in basketball, "nothing but net". :-).

Draft TLO article coming shortly!

Best,
Kate

From: frank lecurieux <[redacted]
Date: Friday 13 March 2015 14:59
To: Kate Guyton <[redacted]
Cc: Andy Shapiro <[redacted]>, Matthew Ross [redacted], Matt Martin <[redacted]>, Lauren Zeise <[redacted]>, "Rusyn, Ivan" <[redacted]
Subject: RE: Thanks!

Dear Kate, all,

Thanks for the dream-team qualification, that I appreciate particularly as a former basket-ball player ☺

There is **one reflection** I had after the plenary session on Tuesday, that I would like to share with you:
Considering the key role that the conclusion of sub-group 4 (mechanisms) may now have in some cases (e.g. for upgrading from 2A to 2B), I believe it may be beneficial if sub-group 1 (exposure) would be involved at some point, and possibly before the plenary, in the analysis of the data generated (in vivo) in humans. I am referring to the plenary discussion we had on genotoxicity studies on humans for glyphosate (formulation). But this may also apply to other endpoints. Hope this may be helpful.

Cheers,
Frank

From: Kathryn Guyton [redacted]
Sent: 13 March 2015 12:20
To: LE CURIEUX Frank; Matthew Ross; Matt Martin; Lauren Zeise; Rusyn, Ivan
Cc: Andy Shapiro
Subject: Thanks!

005430

Dear Frank,

Thank you for your kind words, and for the (fuzzy) pictures! It was wonderful to have you all in Lyon and I'm glad we managed to have at least one relaxing evening together. Many thanks to Ivan for hosting!

In addition to being the Subgroup 4 "dream team" (Kurt's words!) I also wanted to thank you for your outstanding contributions during the Plenary discussion. We were all impressed that Matt(s) Martin was able to quickly calculate p values for the C-A trend test to aid interpretation of the bioassay data! Moreover, recognising the importance of such analyses for interpretation, Andy is busy incorporating standard statistical analyses that would be run in the IARC Table Builder for all entered bioassay incidence data. The pairwise (Fischer) and trend (Cochran-Armitage) tests would thus be automatically run, albeit it will still be possible to enter results of other analyses (e.g., Poly-3 if survival adjustment is possible). I'll be happy to share this when Andy is ready, and welcome your feedback.

Meantime, we've been hard at work drafting the Lancet Oncology article. I'l send it around to you all soon in a google doc (thank you for that suggestion, Matt!). You can also provide input on a Word file. Comments due Monday COB your time.

Hope you all had a very safe return and that re-entry is going well!
Best,
Kate

From: frank lecurieux <[redacted]
Date: Friday 13 March 2015 08:16
To: Matthew Ross <[redacted]>, Kate Guyton <[redacted]>, Matt Martin <[redacted]>, Lauren Zeise <[redacted]>, "Rusyn, Ivan" <[redacted]
Subject: RE: DZN and GLY: section 6 from sub-group 4

Dear all,

First, may I repeat that it was a real pleasure to meet and work with you for IARC monograph vol 112. I think we made quite a nice team – Thanks ☺

Thanks also for the nice moments we shared during the (little) free time we had in Lyon. As promised, here are two photos taken at Ivan's place on Monday evening. The quality of the photos is not so good but I believe the nice atmosphere of the evening clearly shines through the photos ...
[please forward the photos to Andy, as I don't have his e-mail address]

Greetings from a sunny but chilly (0 deg celcius) Helsinki,
Take care

005431

Frank

Frank Le Curieux
Evaluation - E3
European Chemicals Agency
Annankatu 18, P.O. Box 400, FI-00121 Helsinki, Finland
[redacted]
http://echa.europa.eu

003123

From: Kathryn Guyton
To: Ross, Matthew; Ivan Rusyn; Lauren Zeise; Martin, Matt; [redacted] Jahnke, Gloria (NIH/NIEHS) [E]; Calaf, Gloria
Cc: Kurt Straif; Dana Loomis
Subject: Glyphosate- information requests
Date: Friday, April 1, 2016 7:02:10 AM

Dear Vol 112 Working Group members,

It has been brought to our attention that two state universities in the US have received information requests, issued under US state open records laws, concerning the IARC evaluation of glyphosate. IARC is not in a position to offer legal advice to you or your institution concerning these requests. However, it is the position of IARC that all draft documents and materials prepared by the Working Group in advance of or during the in-person Monograph meeting are to be considered draft and deliberative. Working Group members prepare these materials on behalf of IARC, and not as part of their official employment duties for a state or federal institution, and IARC is the sole owner of all such materials. IARC does not encourage participants to retain working drafts of documents after the related Monograph has been published.

We hope this information is helpful to you.

With kind regards,
Kate

Kate Z. Guyton PhD DABT
Responsible Officer, Volume 112
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004095
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World Health Organization

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Office of the Director of
Administration and Finance
[redacted]
http://www.iarc.fr

Ref.: IMO/75/1/-0
vv/as

07 April 2016

Dear Working Group Members,

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 112: Some Organophosphate Insecticides and Herbicides: Diazinon, Glyphosate, Malathion, Parathion, and Tetrachlorvinphos

It has come to our attention that some members of the Working Group of the above-mentioned IARC Monographs Volume 112, or their institutes, received requests for disclosure of documents relating to their work as members of the Working Group.

As a member of the Working Group, we would like to bring to your attention that all documents in your possession, or your institute's possession, relating to your work as a member of this Working Group are documents of the International Agency for Research on Cancer (IARC).

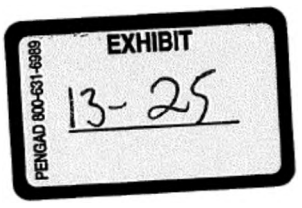
This is also to inform you that, taking into account the status of IARC, which is a part of the World Health Organization (WHO) – an international organization established by treaty and subject to international law – any disclosure of IARC documents in your, or your institute's possession, including any related communications, would be contrary to its privileges and immunities. Moreover, insofar as any such document is a draft document or contains comments on draft documents, these are not intended for further circulation or citation. Furthermore, disclosure of information about the contribution of individual experts (including all members of the Working Group) to the Monographs Volume 112 and any related communications would be prejudicial to the work of IARC/WHO. The development of monographs requires the free and confidential exchange of views and information, bearing also in mind that the entire monograph is the joint product of a Working Group and there are no individually authored sections.

For all of the above reasons, IARC requests you and your institute to not release any documents in your, or your institute's possession relating to your work in the capacity as a member of the Working Group. Should you or institute have any doubt, please contact us – or please ask your institute to contact us - urgently by email to imo@iarc.fr, before responding to any request for disclosure of IARC documents.

Thank you for your cooperation.

Yours faithfully,

Angkana Santhiprechachit
Director of Administration and Finance, ad interim



IARC Monographs on the Evaluation of Carcinogenic Risks to Humans
VOLUME 112: SOME ORGANOPHOSPHATE INSECTICIDES AND HERBICIDES:
DIAZINON, GLYPHOSATE, MALATHION, PARATHION, AND TETRACHLORVINPHOS
Lyon, France: 3-10 March 2015

LIST OF PARTICIPANTS

Working Group Members and Invited Specialists served in their individual capacities as scientists and not as representatives of their government or any organization with which they are affiliated. Affiliations are provided for identification purposes only.

Members

Isabelle Baldi, University of Bordeaux, France
Aaron Blair, National Cancer Institute, USA [retired] (Overall Chair)
Gloria M. Calaf, Tarapaca University, Chile
Peter P. Egeghy, U.S. Environmental Protection Agency, USA[1] (Unable to attend)
Francesco Forastiere, Regional Health Service of the Lazio Region, Italy (Subgroup Chair, Cancer in Humans)
Lin Fritschi, Curtin University, Australia (Subgroup Chair, Exposure)
Gloria D. Jahnke, National Institute of the Environmental Health Sciences, USA
Charles W. Jameson, CWJ Consulting, LLC, USA (Subgroup Chair, Cancer in Experimental Animals)
Hans Kromhout, Utrecht University, The Netherlands
Frank Le Curieux, European Chemicals Agency, Finland
Matthew T. Martin, U.S. Environmental Protection Agency, USA
John McLaughlin, University of Toronto, Canada
Teresa Rodriguez, National Autonomous University of Nicaragua, Nicaragua (Unable to attend)
Matthew K. Ross, Mississippi State University, USA
Ivan I. Rusyn, Texas A&M University, USA (Subgroup Chair, Mechanisms)
Consolato Maria Sergi, University of Alberta, Canada
Andrea 't Mannetje, Massey University, New Zealand
Lauren Zeise, California Environmental Protection Agency, USA

Invited Specialists

Christopher J. Portier, Agency for Toxic Substances and Disease Registry, USA [retired][2]

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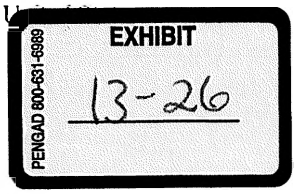
[1] Peter P Egeghy received "in kind" support and reimbursement of travel expenses of on average less than US $2.000 per year during the last 4 years from participation in meetings sponsored by the American Chemistry Council, an industry trade association for American chemical companies, and the Health and Environmental Sciences Institue (HESI), a nonprofit scientific research organization based in Washington and funded by corporate sponsors.

[2] Christopher J Portier receives a part-time salary from the Environmental Defense Fund, a U[illegible] based nonprofit environmental advocacy group.



IARC Monographs on the Evaluation of Carcinogenic Risks to Humans
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Lyon, France: 3-10 March 2015

Representatives of national and international health agencies

Amira Ben Amara, National Agency for Sanitary and Environmental Product Control, Tunisia (Unable to attend)
Catherine Eiden, U.S. Environmental Protection Agency, USA (Unable to attend)
Marie-Estelle Gouze, for the French Agency for Food, Environment and Occupational Health and Safety, France
Jesudosh Rowland, U.S. Environmental Protection Agency, USA

Observers

Mette Kirstine Boye Jensen, for Cheminova A/S, Denmark[3]
Béatrice Fervers, for the Léon Bérard Centre, France
Elodie Giroux, University Jean-Moulin Lyon 3, France
Thomas Sorahan, for Monsanto Company, USA[4]
Christian Strupp, for the European Crop Protection Association, Belgium[5]
Patrice Sutton, for the University of California, San Francisco, Program on Reproductive Health and the Environment, USA[6]

IARC secretariat

Lamia Benbrahim-Tallaa, Section of *IARC Monographs*
Rafael Carel, Visiting Scientist, University of Haifa, Israel, Section of *IARC Monographs*
Fatiha El Ghissassi, Section of *IARC Monographs*
Sonia El-Zaemey, Section of the Environment and Radiation
Yann Grosse, Section of *IARC Monographs*
Neela Guha, Section of *IARC Monographs*
Kathryn Guyton, Section of *IARC Monographs (Responsible Officer)*
Charlotte Le Cornet, Section of the Environment and Radiation
Maria Leon Roux, Section of the Environment and Radiation

---

[3] Mette Kristine Boye Kristensen is employed by Cheminova A/S, Denmark, a global company developing, producing and marketing crop protection products.

[4] Tom Sorahan is a member of the European Glyphosphate Toxicology Advisory Panel, and received reimbursement of travel cost from Monsanto to attend EuroTox 2012.

[5] Christian Strupp is employed by ADAMA Agricultural Solutions Ltd, Israel, a producer of Diazinone and Glyphosphate.

[6] Patrice Sutton's attendance of this Monographs meeting is supported by the Clarence E. Heller Charitable Foundation, a philanthropic charity with a mission to protect and improve the quality of life through support of programs in the environment, human health, education and the arts.

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans
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Lyon, France: 3-10 March 2015

Dana Loomis, Section of *IARC Monographs*
Heidi Mattock, Section of *IARC Monographs (Editor)*
Chiara Scoccianti, Section of *IARC Monographs*
Andy Shapiro, Visiting Scientist, Section of *IARC Monographs*
Kurt Straif, Section of *IARC Monographs (Section Head)*
Jiri Zavadil, Section of Mechanisms of Carcinogenesis

NOTE REGARDING CONFLICTS OF INTERESTS: Each participant submitted WHO's Declaration of Interests, which covers employment and consulting activities, individual and institutional research support, and other financial interests. Participants identified as Invited Specialists did not serve as meeting chair or subgroup chair, draft text that pertains to the description or interpretation of cancer data, or participate in the evaluations. The Declarations were updated and reviewed again at the opening of the meeting.

NOTE REGARDING OBSERVERS: Each Observer agreed to respect the Guidelines for Observers at *IARC Monographs* meetings. Observers did not serve as meeting chair or subgroup chair, draft any part of a *Monograph*, or participate in the evaluations. They also agreed not to contact participants before the meeting, not to lobby them at any time, not to send them written materials, and not to offer them meals or other favours. IARC asked and reminded Working Group Members to report any contact or attempt to influence that they may have encountered, either before or during the meeting.

Posted on 26 January 2015, updated 19 October 2016

HEYDENS, WILLIAM F [AG/1000]

From: Thomas Sorahan [redacted]
Sent: Saturday, March 14, 2015 6:18 AM
To: FARMER, DONNA R [AG/1000]; Strupp Christian; Mette K. Jensen
Cc: HEYDENS, WILLIAM F [AG/1000]
Subject: RE: EPA openly discussed IARC findings at a CLA meeting on Thursday

Dear Donna

I understand your concerns about early release of information. We can discuss the issues you raise in more detail on Monday, but here are some immediate responses.

I do know of instances where observers at IARC felt they had been treated rudely or brusquely at Monograph meetings. That was not the case for me at Vol 112. I found the Chair, sub-chairs and invited experts to be very friendly and prepared to respond to all comments I made. Indeed, I think questions the epi sub-panel asked me about my recent multiple myeloma paper (Sorahan, 2015) were instrumental in not having multiple myeloma included on the charge sheet.

In my opinion the meeting followed the IARC guidelines. Dr Kurt Straif, the Director of the Monographs programme, has an intimate knowledge of the IARC rules and insists these are followed.

As you say, there are background sections in the Monograph preambles and presumably on the IARC website as to how the IARC process is supposed to work. The recent EHP paper you have by Pearce et al (the 124 author effort) is also good for describing how things are supposed to work (about the only thing it is good for).

I suppose the main difference between IARC evaluations and most national agency guidelines is that IARC has nothing to say (directly) about potency and appropriate exposure limits.

As you know, the Working Group (WG) only has four choices for evaluating the human data (evidence of no carcinogenicity [in practice, protective effect], inadequate, limited, sufficient). The WG chose limited for NHL and glyphosate, but it is not clearly laid down what is the difference between the upper band of inadequate and the lower band of limited. As far as I can see, this is left to each WG to decide on its own.

These remarks are all confidential and I do not wish to be referenced in any document from your PA/PR people. But I am happy to assist in formulating statements that you may wish to make (eg "The company does not accept there is credible evidence that glyphosate use can cause NHL. Indeed in the single most important study into the health of pesticide applicators (the AHS) there is no excess of NHL in all applicators when compared to State cancer incidence rates, no excess in glyphosate users compared to non-users, and no trend of NHL increasing with extent of use"). I'm sure Elizabeth Delzell will be going into some detail in comparing the NHL findings from the case-control studies and from the AHS, in her proposed meta-analysis.

Tom

-----Original Message-----
From: FARMER, DONNA R [AG/1000] [redacted]
Sent: 14 March 2015 02:25
To: Thomas Sorahan; Strupp Christian; Mette K. Jensen
Cc: HEYDENS, WILLIAM F [AG/1000]
Subject: EPA openly discussed IARC findings at a CLA meeting on Thursday

Tom, Christian and Mette,

One of our colleagues was on a CLA call with other companies, EPA and PRMA for the Residue Experts Work Group at the DOW office yesterday. The EPA person opened the meeting by telling the group that an EPA Observer (Jess Rowland) was in the meeting, reported back to EPA Staff that IARC classified 3 pesticides as 2a and then he named diazinon, malathion and glyphosate. When asked by our colleague that it was our understanding that that information was under embargo wasn't that his understanding as well...he said he was not told to keep the information embargoed. The EPA person said the EPA is not IARC, he was providing this report, without comment. The subject was not on the agenda; he offered up without asking.

Mutagenesis vol. 26 no. 1 pp. 19–26, 2011 doi:10.1093/mutage/geq070

REVIEW

Micronuclei and pesticide exposure

Claudia Bolognesi, Amadeu Creus[1,2], Patricia Ostrosky-Wegman[3] and Ricard Marcos[1,2,*]

Environmental Carcinogenesis Unit, Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy, [1]Grup de Mutagènesi, Departament de Genètica i de Microbiologia, Facultat de Biociències, Edifici Cn, Universitat Autònoma de Barcelona, 08193 Bellaterra, Cerdanyola del Vallès, Spain, [2]CIBER Epidemiología y Salud Pública, ISCIII, Spain and [3]Department of Genomic Medicine and Environmental Toxicology, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma De México, México

*To whom correspondence should be addressed. Grup de Mutagènesi, Departament de Genètica i de Microbiologia, Facultat de Biociències, Edifici Cn, Universitat Autònoma de Barcelona, 08193 Bellaterra, Cerdanyola del Vallès, Spain. Tel: +34 935812052; Fax: +34 935812387; Email: ricard.marcos@uab.es

Received on June 14, 2010; revised on July 28, 2010; accepted on August 25, 2010

Micronucleus (MN) is a biomarker widely used in biomonitoring studies carried out to determine the genetic risk associated to pesticide exposure. Many *in vitro* and *in vivo* studies, as well as epidemiological approaches, have demonstrated the ability of certain chemical pesticides to produce genetic effects including cancer and other chronic pathologies in humans; thus, biomonitoring studies have been carried out to characterise the genetic risk associated to pesticide exposure. It must be noted that 'pesticide exposure' is a broad term covering complex mixtures of chemicals and many variables that can reduce or potentiate their risk. In addition, there are large differences in pesticides used in the different parts of the world. Although pesticides constitute a wide group of environmental pollutants, the main focus on their risk has been addressed to people using pesticides in their working places, at the chemical industry or in the crop fields. Here, we present a brief review of biomonitoring studies carried out in people occupationally exposed to pesticides and that use MN in lymphocytes or buccal cells as a target to determine the induction of genotoxic damage. Thus, people working in the chemical industry producing pesticides, people spraying pesticides and people dedicated to floriculture or agricultural works in general are the subject of specific sections. MN is a valuable genotoxic end point when clear exposure conditions exist like in pesticide production workers; nevertheless, better study designs are needed to overcome the uncertainty in exposure, genetic susceptibility and statistical power in the studies of sprayers and floriculture or agricultural workers.

Introduction

A large number of synthetic pesticides have been introduced in the market since the mid-1940s. At present, the pesticide manual includes 900 main entries and lists over 2600 products (1). Pesticides, as a heterogeneous category of biologically active compounds, are characterised by various degrees of toxicity also to non-target species, including human beings. Most pesticides are acutely toxic to humans. Cases of acute pesticide poisonings account for significant morbidity and mortality worldwide, especially in developing countries, where the pattern of pesticide use is different (2,3).

Chronic health effects have been associated to pesticide exposure, including neurological effects, reproductive or development problems and cancer. Epidemiological studies on farmers, pesticide manufacturers, pesticide sprayers and on accidentally exposed industrial workers or residents have shown that exposure to pesticides may increase the risk of site-specific cancers. Increased risks have been detected for brain cancer, leukaemia and Ewing's bone sarcomas, kidney cancer, acute leukaemia, soft tissue sarcoma, non-Hodgkin's lymphoma, brain cancer, testicular, colorectal, endocrine glands and brain cancers in children exposed to pesticides in their home or whose parents were occupationally exposed to pesticides (4). Reproductive effects (5,6), developmental problems and very recently neurodegenerative disorders, such as Parkinson (7,8) and Alzheimer disease (9,10), have been also associated to occupational exposure to pesticides. Many pesticides involved in carcinogenic risk, and classified as probable or possible carcinogens by the International Agencies, were banned or their use was restricted in some countries; but, due to their bioaccumulation and persistence in the ecosystems, they are widespread environmental pollutants. Residues of these pesticides have been detected in the food chain and in different biological media in humans.

At present, the regulations concerning the introduction of plant protection products on the market in the developed countries (e.g. Dir. 91/414/EEC, EPA Regulations) involve the evaluation of all the active substances in a pesticide product. Pesticides containing substances that are carcinogenic (except for those with a threshold mode of action) and/or genotoxic are not allowed to be placed on the market and for already authorised compounds, if new data become available showing that the substances may have these potentials, they will be withdrawn from the market. Acute and chronic effects are determined by observing symptoms in test animals, resulting from lifetime exposure to the active substances. However, delayed adverse health effects can be often identified or confirmed only through epidemiological studies in occupationally exposed populations.

Genotoxicity risk of pesticide exposure

Genotoxic potential is a primary risk factor for long-term effects, such as carcinogenic and reproductive toxicology and degenerative diseases. Biomonitoring studies focusing on genomic modifications have been carried out in pesticide-exposed populations from different countries to elucidate the risk associated to the exposure to specific compounds or classes of compounds or to specific cultivation practices (11,12).

Among them, several studies employing the micronucleus (MN) test in peripheral lymphocytes or in exfoliated buccal mucosa cells are available in the last decades. Occupational exposure is the normal source of information on the risk associated to pesticide exposure. Nevertheless, this exposure usually involves complex mixtures of pesticides belonging to different chemical classes varying with the type of crop, the season and the geographical area

Taken into account the complexity of these exposures, in this review, we have structured the studies applying the MN test in peripheral blood lymphocytes (PBL) according to the following topics: (i) results obtained in people working in the chemical industry producing pesticides, (ii) studies on pesticide sprayers, (iii) studies in floriculturists and (iv) studies in agricultural workers not included in the previous sections. A further section (v) includes studies that have used the MN assay in buccal cells.

MN in PBL of workers from pesticide industries

The available studies on workers from pesticide industries showed a statistically significant increase of MN frequency in PBL (Table I). The MN frequency in 41 workers exposed to chlorinated compounds, including hexachlorobenzene (HCB) in Sao Paulo (Brazil), is significantly higher than in controls, showing also a correlation with working time and with serum concentration of HCB (13). Two studies carried out in Croatia in workers exposed to 2,4-dichlorophenoxyacetic acid (2,4-D), atrazine, alachlor, cyanazine and malathion, during the process of production, show significant increases in the MN frequency after 8 months of high exposure (14,15). In a recent study carried out in Pakistan with workers from an industry producing pesticides, belonging to the organophosphate and pyrethroid classes, significant increases in the MN frequency were observed in workers, showing a linear correlation with length of exposure (16).

MN in the PBL of pesticide sprayers

Pesticides sprayers are directly involved in treating specific pests by spraying/fumigating the crops and represent the most exposed group among the agricultural workers. Among sprayers, we can find workers applying specifically one or few pesticides, while others use mixtures of pesticides. The biomonitoring studies concerning the use of one or few pesticides are all related to professional applicators working under controlled conditions: no increase in chromosomal damage was observed (Table IIa). The frequency of MN in a group of 31 fumigators of commercial grain stores in Australia using phosphine was not significantly different to that observed in controls, indicating a lack of genotoxic risk keeping low levels (2.4 p.p.m./h) of exposure (17).

Two studies were conducted in California (USA) with workers involved in the Mediterranean Fruit Fly Eradication Program. In 38 intermittently malathion-exposed sprayers, no increase in the frequency of MN in PBL was detected (18). In a second study, a slight but significant increase in the MN frequency was observed in workers exposed to malathion for >50 h during the last 8 months or with levels of malathion diacid >100 p.p.b. (19).

Methyl bromide fumigators have also been the subject of a biomonitoring study testing the levels of MN in lymphocytes (20). This study was carried out in USA and no increases were observed in the MN frequency of fumigators. These negative findings contrast with those observed in the same group of workers, when the frequency of MN was measured in oropharyngeal cells and when hypoxanthine–guanine phosphoribosyl transferase gene (*HPRT*) mutations were measured in lymphocytes.

No genotoxic risk was associated to the herbicide 2,4-D exposure as evaluated in a group of sprayers from eastern Kansas (USA): no significant difference in MN frequency was observed between workers and controls and before and after the spraying period (21). A biomonitoring study carried out with 11 fumigators at the tobacco fields in western Greece, using metalaxil as fungicide and imidacloprid as insecticide, did not show any significant increase in the frequency of MN in PBL (22).

The studies carried out with sprayers applying complex mixtures of pesticide (Table IIb) include heterogeneous populations involved in cultivation of different crops, in sanitisation and indirectly exposed by aerial spraying. Four of five studies give positive results. Significant increases of MN associated to the duration of exposure were observed in a study carried out with sprayers from central Italy (23). A study conducted in vineyards workers from Serbia, applying mainly insecticides and fungicides, showed higher MN frequency compared to controls 1 month after the start of the spraying period, with a further increase at the end of the spraying season (24). No significant effects were observed in workers from Concepción (Chile), who sprayed a variety of pesticides, mainly the insecticides deltamethrin and dichlorvos (25).

Positive effects were also reported in a group of sanitation workers from Belo Horizonte (Brazil), using different pesticides including organophosphates and pyrethroid insecticides, as well as hydroxycoumarinic rodenticides. No time exposure association was found (26).

Table I. Biomonitoring studies using peripheral blood lymphocytes from human populations exposed to pesticides: MN in chemical plant workers

Study subjects/ controls	Exposure (chemicals)	Duration (years)	Result (fold difference versus controls)	PPE	Time dependence	Country	Reference
41/28	Chlorinated compounds, including HCB	9	Pos (+3.6)	NA	No evaluated	Brazil	da Silva Augusto *et al.* (13)
20/20	Pesticide production limited to 8 months/year (2,4-D, atrazine, alachlor, cyanazine, malathion)	4–30	Pos; after 8 months of high exposure (+3.63), after 8 months of non-exposure (+1.86)	NA	Yes	Croatia	Garaj-Vrhovac and Zeljezic (14)
10/20	Pesticide production limited to 8 months/year (2,4-D, atrazine, alachlor, cyanazine, malathion)	4 30	Pos (+7.9)	NA	No evaluated	Croatia	Garaj-Vrhovac and Zeljezic (15)
35/29	Complex mixtures, mainly organophosphates and pyrethroids	3–18	Pos (+2.06)	No	Yes	Pakistan	Bhalli *et al.* (16)

NA, not available; PPE, personal protective equipment.

Table II. Biomonitoring studies using peripheral blood lymphocytes from human populations exposed to pesticides: MN in pesticide sprayers

Study subjects/ controls	Exposure (chemicals)	Duration (years)	Result (fold difference versus controls)	PPE	Time dependence	Country	Reference
a) Exposure to single pesticide							
31/21	Fumigators: phosphine (2.4 p.p.m. in enclosed spaces)	1.5–32	Neg	NA	NA	Australia	Barbosa and Bonin (17)
38/16	Medfly eradication programme: malathion, exposure below the genotoxic dose	NA	Neg, after spraying season (no correlation with metabolites in urine)	NA	NA	USA	Titenko-Holland *et al.* (18)
1992 cohort, 13/4, 1993 cohort, 24/10	Medfly eradication programme: malathion fumigations	NA	Pos (+1.4), malathion diacid, >100 p.p.b. in urine (+1.58), Neg	NA	NA	USA	Windham *et al.* (19)
31/27	Fumigant appliers: methyl bromide	0.3–22	Neg	NA	No	USA	Calvert *et al.* (20)
12/9	Pesticide applicators: 2,4-D (240 + 100 p.p.b.), 12–1285 p.p.b.	Discontinuous use	Before and after Neg	Yes	No	USA	Figgs *et al.* (21)
11/11	Tobacco fields sprayers using metalaxyl and imidacloprid	23.64 ± 4.13	Neg	Yes (50%)	No	Greece	Vlastos *et al.* (22)
b) Exposure to mixture of pesticides							
48/50	Farmers (cereals, fruits, vegetables): pesticide mixture	4–50	Pos (+1.20)	Yes (29%)	Pos	Italy	Pasquini *et al.* (23)
27/20	Vineyard workers: pesticides most used: diazinon and dithiocarbamate	12.1	Pos (+7.67) end of spraying season	NA	Pos (P = 0.016)	Serbia	Joksic *et al.* (24)
22/16	Pesticides most used: bromadialone, captan, deltamethrin, diazinon dichlorvos, linuron, methamidophos	7	Neg	NA	NA	Chile	Venegas *et al.* (25)
29/30	Sanitation workers. Complex mixtures and types of application	23.64 ± 4.1 (1.5–18)	Pos (+3.35)	Yes	Neg	Brazil	Kehdy *et al.* (26)
62/60	Pesticide mixture	NA	Pos (+2.71)	NA	NA	Colombia	Bolognesi *et al.* (27)
60/60	Glyphosate aerial spraying for control of illicit crops		Pos (+2.53)				
64/60			Pos (+3.26)				
28/60	Glyphosate aerial spraying for sugar cane maturation		Pos (+4.72)				

Neg, negative; Pos, positive; PPE, personal protective equipment.

A recent study was carried out in Colombia to investigate the health effects associated with glyphosate exposure, in the aerial spraying programme for control of illicit crops and in the maturation of sugar cane in comparison with the exposure to pesticide mixture (27). In regions where glyphosate was being sprayed, blood samples were collected prior, during and 4 months after spraying. Results showed significant increases in MN frequency after glyphosate exposure, mainly when it is applied for maturation of sugar cane.

MN in PBL of floriculturists

Floriculturists are involved in the production of flowers and ornamental plants, which are commonly treated with high quantities of agrochemical formulations in greenhouses.

Several studies have been carried out with this collective (Table III), mainly in Italy, where in 1993, one study was performed in the region of Liguria (Northwest of Italy). This study carried out with 71 workers showed significant increases in the frequency of MN in people occupationally exposed to pesticides. The MN frequency showed a dose–response relationship with duration of exposure, with a maximum increment of 71% in the MN frequency in subjects exposed for over 30 years (28,29). Further studies in this population indicated that the conditions of exposure influenced the MN frequency. Thus, increased relative risks (RR) in greenhouse workers (RR = 1.31) and in people working alternately in the greenhouse and in the open field (RR = 1.46) were observed with respect to the reference population (30).

A further study in the same area and by the same group was carried out in workers producing ornamental plants and vegetables. A statistically significant increase in the MN of 107 floriculturists was detected with respect to the control population, and a positive correlation between years of farming and MN frequency was observed. The conditions of exposure were also associated with an increase in cytogenetic damage, with a 28% higher MN frequency in greenhouse workers compared with subjects working only in open fields. Finally, workers not using protective measures during high exposure activities showed an increase in the MN frequency (34).

To determine the mechanisms producing MN, 52 floriculturists and 24 controls were evaluated by using the cytokinesis-block methodology associated with fluorescence *in situ* hybridisation with a pan-centromeric probe that allowed distinguishing centromere-positive (C+) and centromere-negative (C−) MN. The percentage of C+ MN was not related to the duration of exposure or to the number of genotoxic pesticides used, but a higher percentage (66.52 versus 63.78%) was observed in a subgroup of subjects using benzimidazolic compounds compared with the floriculturist population exposed to a complex pesticide mixture not including benzimidazolics (35).

Two other studies including floriculturists were carried in Tuscany (Central Italy). In this area, floriculturists used many different formulations and performed two types of

Table III. Biomonitoring studies using peripheral blood lymphocytes from human populations exposed to pesticides: MN in floriculturists

Study subjects/ controls	Exposure (chemicals)	Duration (years)	Result (fold difference versus controls)	PPE	Time dependence	Country	Reference
71/75	Complex pesticide mixtures	2–55	Pos (1.29)	Yes	Yes	Italy	Bolognesi *et al.* (28–30)
43/41	Greenhouse workers: >100 agrochemical formulations	NA	Neg	NA	NA	Italy	Scarpato *et al.* (31)
23/22	Greenhouses using: benzimidazoles, carbamates, diphenylethanoles, dithiocarbamates, organophosphates, thiophthalimides	NA	Neg	Yes	NA	Italy	Scarpato *et al.* (32)
34/33, 17/— highly exposed sprayers	Greenhouse workers: complex mixture of pesticides	7–41	Neg, Pos (+1.22)	Yes	NA	Italy	Falck *et al.* (33)
107/61	Greenhouse and open field workers	2–70	Pos (+1.45), greehouses/open field (+1.22), No PPE/PPE (+1.17)	Yes (15%)	Yes	Italy	Bolognesi *et al.* (34)
51/24	Greenhouses (80%) and open field (20%) using >50 different pesticides	26.3 ± 14.5	Neg	NA	Yes	Italy	Bolognesi *et al.* (35)
31/30	Women field workers, complex mixtures	10.97 (2–22)	Pos	Yes (49.2%)	No	Colombia	Varona *et al.* (36)

Neg, negative; Pos, positive; PPE, personal protective equipment.

work: culture treatment (mixing and spraying of pesticides) or re-entry activities (cutting and harvesting flowers several hours after the end of pesticide spraying). MN frequency in PBL from the floriculturists did not show differences compared with controls (31). Blood samples obtained during and 1 month after the end of intensive pesticide treatments were analysed to cover a period of high and low exposure, respectively, but no effect of pesticide exposure was detected. Each donor was genotyped for polymorphisms in the *GSTM1*, *GSTT1* and *NAT2* genes, involved in xenobiotic metabolism, but no association was observed between MN frequency and the genetic polymorphisms analysed (32). Nevertheless, a subsequent study showed that *GSTM1* positive and *NAT2* fast appear associated to MN increases (33). Finally, a study carried out in Colombia with women working in open fields observed significant increases in MN associated to pesticide exposure (36).

MN in PBL of agricultural workers

A survey of studies carried out in agricultural workers is shown in Table IV. A first study was carried out in Italy with open field and greenhouse workers exposed to complex pesticide mixtures, but no effects were detected (37). Negative results were also obtained in seasonal farm workers from British Columbia (Canada) harvesting berry crops. Subjects were 39 females of South Asian descent, 18 farm workers and 21 age-matched controls. Interestingly, the highest frequency of MN cells was found in the group with the longest history of employment as a farm worker. In addition, farm workers had a lower frequency of kinetochore-positive MN than controls (38).

Two studies were carried out in the south-eastern of Spain. PBL samples from 64 workers exposed to complex mixtures of pesticides did not show any increase in the frequency of MN. This lack of genotoxic effects did not change when agricultural workers were classified according their genotypes for *GSTM1* and *GSTT1* (39). A follow-up study, carried out with 39 greenhouse workers from the same group, compared the effects of high exposure (spring–summer) and lower exposure (autumn–winter). Results indicated that no statistically significant differences in the MN frequencies were found neither between the two sampling periods nor between the exposed and controls (44).

The same research group carried out three different studies with three other European populations in Poland, Greece and Hungary. Neither the Poland group (49 subjects) nor the Greece (50 workers) and the Hungarian group (84 workers) presented significant increases in MN frequency in their PBL (41–43). In spite of this lack of genotoxic effects, decreases in the cell proliferation index were observed, indicating some type of effect related to pesticide exposure. A summing up study was carried out with the above-cited populations, including 239 agricultural workers and 231 unexposed controls. The results indicated that, for the overall population, there were no increases in MN frequencies in the agricultural workers when compared with the controls (45).

In a study carried out in Costa Rica in banana farms, no increases in MN frequency were observed in women, exposed for at least 4 months to the commonly applied compounds imalzalile, thiabendazole and chlorpyriphos. Nevertheless, women with a high frequency of abortions showed increased frequencies of MN (40).

The Bío-Bío Region is a major fruit-growing area of Chile that makes intensive use of agricultural pesticides. In a group of 64 females harvesting and packing different significant increases in MN frequency were found without correlation with the duration of exposure (46). A statistically significant increase in MN frequencies was observed in a small group of 11 agricultural workers growing vineyards and olive trees in Crete (Greece) and exposed to complex mixtures of pesticides (47).

A study with 15 agricultural workers from Kentucky (USA), exposed for 6 months to several pesticides, showed a 76% increase in the average MN frequency in lymphocytes. In addition, MN frequency peaked during the period of highest exposure (48). In a biomonitoring study with 28 agricultural workers from the region of the Atoyac River (Mexico), increase in the MN frequency was observed, with higher values

Table IV. Biomonitoring studies using peripheral blood lymphocytes from human populations exposed to pesticides: MN in agricultural workers

Study subjects/ controls	Exposure (chemicals)	Duration (years)	Result (fold difference versus controls)	PPE	Time dependence	Country	Reference
62/29	Open field and greenhouse workers. Complex pesticide mixtures	2–52	Neg	NA	Yes	Italy	Bolognesi *et al.* (37)
18/21	Berry pickers exposed mainly to simizine, paraquat, napropamide, glyphosphate captan, triforine, diazinon, malathion, carbofuran, endosulfan	1–24	Neg	NA	Yes	Canada	Davies *et al.* (38)
64/50	Greenhouse workers. Complex pesticide mixture	9.82 ± 1.0	Neg	Yes (80%)	No	Spain	Lucero *et al.* (39)
32/37	Banana farms. Imalzalile and thiabenzadole (fungicides) and chlorpyriphos (insecticide)	>4 consecutive months	Neg	NA	No	Costa Rica	Ramírez and Cuenca (40)
49/50	Greenhouse and open field: vegetables and ornamental plants	16.28 ± 1.1	Neg	Yes (78%)	NA	Poland	Pastor *et al.* (41)
50/66	Open field: vegetables and ornamental plants	8.62 ± 1.13	Neg	Yes (62%)	NA	Greece	Pastor *et al.* (42)
84/65	Open field/greenhouse workers: pesticide mixture	18.75 ± 0.89	Neg	Yes (85%)	NA	Hungary	Pastor *et al.* (43)
39/22	Greenhouse workers	8.31 ± 1.12	Neg	Yes (93%)	No	Spain	Pastor *et al.* (44)
239/231	Open field/greenhouses. Complex pesticide mixtures	13.92 ± 0.58	Neg	Yes	No	Spain, Greece, Hungary, Poland	Pastor *et al.* (45)
64/30	Thinning and pruning fruit trees, harvesting and packaging fruits	8 ± 4.8	Pos (+3.72)	No	NO	Chile	Márquez *et al.* (46)
11/11	Vineyards and olive tree cultures. Organophosphates and pyrethroids, the most used	26.45 ± 3.38 (25–60)	Pos (+1.40)	NA	NA	Greece	Vlastos *et al.* (47)
15/10	Complex mixtures including endosulfan, chlorpyriphos, dimethoate, diazinon and maleic hydrazide	18.2 ± 1.3	Pos (+1.76)	NA	NA	USA	Tope *et al.* (48)
28/21	Polluted areas including pesticide-polluted areas	NA	Pos (+1.92)	NA	NA	Mexico	Montero *et al.* (49)
33/33	Open field and greenhouses	15.0 ± 13.0 (0.5–48)	Pos (+2.76), greenhouses/open field, Pos (+1.86)	33% (gloves)	No	Portugal	Costa *et al.* (50, 51)
69/69	Cotton pickers (carbamates, organophosphates, pyrethroids)	10.3 ± 6.1	Pos (+2.92)	NA	Yes	Pakistan	Ali *et al.* (52)
108/65	Open fields: grapes growers	NA	Pos (+1.69)	NA	NA	Brazil	da Silva *et al.* (53)

Neg, negative; Pos, positive; PPE, personal protective equipment.

in people with the *GSTT1* null allele (49). In the area of Oporto (Portugal), a biomonitoring study was conducted in a group of 33 farmers exposed to pesticides. MN frequency was significantly higher in the exposed group and it was possible to relate a specific working environment (greenhouses) with higher levels of genetic damage and the use of personal protective equipments with lower frequencies of MN. No association was found between MN frequency and duration of pesticide exposure and, when the effect of polymorphic genes of xenobiotic-metabolising enzymes (*GSTM1*, *GSTT1*, *GSTP1*, *CYP2E1* and *EPHX1*) was evaluated, results suggest that low microsomal epoxide hydrolase activity as well as *GSTT1*-positive genotype are associated with increased cytogenetic damage (50,51). An increase of MN frequency was also shown in a biomonitoring study with 69 females involved in cotton-picking activity in the Bahawalpur area (Pakistan) (52).

In Caxias do Sul (Brazil), 108 vineyard workers showed high rates of MN than controls. When the subjects were genotyped for *GSTT1*, *GSTM1*, *GSTP1*, *CYP1A1*, *CYP2E1* and *PON*, it was shown that genetic polymorphisms in *PON* modulated the frequency of MN in the exposed group. In addition, some associations between *GSTM1*, *GSTT1* and *CYP2E1* polymorphisms were suggested (53).

A study was performed in the umbilical cord blood of 16 newborns, in an agricultural area in Delicias, Chihuahua, in the North of Mexico characterised by the use of pesticide mixtures (mainly organophosphates) during the summer and autumn spraying cycles. No significant increases in MN were observed in this group compared to 35 controls (not exposed to pesticides), although more babies with a higher MN frequencies were within the pesticide-exposed group (54).

MN in buccal cells of pesticide-exposed workers

Table V summarises the studies on MN in buccal cells. The first study reporting effects in buccal cells was carried out in workers exposed to methyl bromide, where higher but not significant MN frequency was observed (20).

A series of studies were carried out with agricultural workers from four European countries (Spain, Poland, Greece and Hungary). The overall results of this study, including 247 agricultural workers and 231 controls, did not indicate any increase in MN frequency in buccal cells related to pesticide exposure. In the Spanish population, an additional analysis determined that *GSTM1* and *GSTT1* polymorphisms did not modify the MN induction (39,41–43).

Table V. Biomonitoring studies using buccal mucosa cells from human populations exposed to pesticides

Study subjects/ controls	Exposure (chemicals)	Duration (years)	Result (fold difference versus controls)	PPE	Time dependence	Country	Reference
32/28	Methyl bromide (from fumigation)	NA	Neg	NA	No	USA	Calvert *et al.* (20)
64/50	Agricultural workers in greenhouses: tralomethrin	9.82 ± 1.0	Neg	Yes (80%)	No	Spain	Lucero *et al.* (39)
30/30	Floriculturists	1.5–10	Pos (+2.7)	No	NA	México	Gómez-Arroyo *et al.* (56)
49/50	Agricultural workers: open field/greenhouse	16.28 ± 1.1	Neg	Yes (78%)	NA	Poland	Pastor *et al.* (41)
50/66	Agricultural workers: open field—vegetables and ornamental plants	8.62 ± 1.13	Neg	Yes (62%)	No	Greece	Pastor *et al.* (42)
84/65	Agricultural workers open field/greenhouses, pesticide mixtures	18.75 ± 0.89	Neg	Yes (85%)	NA	Hungary	Pastor *et al.* (43)
239/231	Open field/greenhouses. Complex pesticide mixtures	13.92 ± 0.58	Neg	Yes	No	Spain, Greece, Hungary, Poland	Pastor *et al.* (45)
40/44	Women working as banana packing exposed to thiabenzadole and chlorpyrifos	6.4	Neg	NA	No	Costa Rica	Castro *et al.* (61)
54/54	Pesticide manufacturing unit: pyrethroids, organophosphates, carbamates	8.57 (3–13)	Pos (+3.9)	No	Yes	India	Sailaja *et al.* (59)
32/32	People living in a pesticide-contaminated area	34.6 ± 10.5	Pos	NA	NA	Turkey	Ergene *et al.* (57)
70/70	Agricultural workers	7.00 ± 3.95	Pos (+7.64)	No	NA	México	Martínez-Valenzuela *et al.* (58)
29/37	Agricultural workers: soybean growers	16.3 ± 10 (2–35)	Pos (+1.99)	Yes (31%)	No	Brazil	Bortoli *et al.* (60)
37/20	Agricultural workers	25.7 ± 10.1	Neg	67.6	No	Brazil	Remor *et al.* (55)

PPE, personal protective equipment; Neg, negative; Pos, positive.

No increase of MN frequency was detected in a group of 40 women working in banana packing facilities in Costa Rica (56). Negative results were also reported in sprayers from the region of Rio Grande do Sul (Brazil) exposed to a wide number of pesticides, although significant variations in the plasmatic levels of butyrylcholinesterase and δ-aminolevulinic acid dehydratase enzymes indicate that exposure did occur (61). In spite of the negative results above indicated, several studies reported significant MN increases in the buccal cells of workers exposed to pesticides.

In Mexico, a study with 30 subjects working as floriculturists in greenhouses shows an increase in MN frequency in buccal cells (55). A further study in Mexico (Sinaloa State) reported a clear increase in MN frequency in agricultural workers using mainly organophosphates and carbamates without any correlation with age, gender or exposure length to pesticides (59).

A study carried out in Hyderabad (India) in a chemical industry producing organophosphates, carbamates and pyrethroids showed significant increases in the MN frequency in subjects working for >10 years (57). Slight but significant increases in the frequency of MN were also reported in the Göksu Delta region (Turkey), a wetland area with intensive agriculture, where rice, cotton and peanuts are grown all over the year (58).

Significant increases in the frequency of MN were observed in the workers involved in soybean culture in the State of Rio Grande do Sul (Brazil); nevertheless, these increases were not related with the use of protective measures or the time of exposure (60).

Knowledge gaps and road map for future research and improvements

The general pattern in pesticide exposure is the simultaneous use of complex mixtures of chemical compounds that makes difficult to determine the possible synergic/antagonist effects among them. In this context, the appearance of the cytokinesis-block micronucleus assay in 1985 (62), as an easy alternative to the chromosome aberration test, opened the possibility to go further in the knowledge of the genotoxic risk associated to pesticide exposure. Nevertheless, the first biomonitoring study of a human population exposed to pesticides using the MN assay was published in 1993. Since then, an exponential use was not observed since 15 studies were reported between 1993 and 1999, 16 between 2000 and 2004 and 16 between 2005 and 2009. This means that, in spite of its advantages, the MN was not been widely used in the biomonitoring of human populations exposed to pesticides.

Actually, even if a number of studies in subjects exposed to single pesticides, or just to a few compounds, allowed to estimate a genotoxic risk associated to defined chemicals, the large majority of the available studies had not generated the reliable information needed for a risk assessment.

Some studies have an inadequate study design or a low statistical power. However, the main limitations of them are the lack of exposure assessment, information on the pesticide use pattern and the characterisation of the relevant factors modulating the exposure.

Surrogate factors for the exposure, such as pesticide consumption, number of genotoxic pesticides applied and duration of exposure were considered in some studies, where a relationship was observed between increased MN frequency and specific agricultural practices or inadequate working conditions. However, the lack of adequate evaluation of individual exposures severely limited any conclusions in regard to the identification of an active ingredient or occupational task, which are clearly identified as responsible for a genetic risk.

The MN test in its comprehensive application (Cytome) and for its role in predicting cancer risk is a useful tool to estimate the genetic risk from the integrated exposure to complex mixture of chemicals associated to the use of pesticides.

One advantage of the MN is that it makes easy to determine mechanism of action of the compounds through the detection

of the presence of kinetochore or centromere in the MN, as a way to distinguish between clastogenicity and aneugenicity, with relevant implications in risk assessment. These approaches were applied only in few studies (18,20,35), revealing an increase in kinetochore-negative or -positive MN related to the mechanism of action of the pesticides.

Further studies should be done in groups of subjects adequately characterised for the exposure in order to define the role of the MN test in pesticide risk assessment. Alternative methods have to be considered to estimate the exposure: the evaluation of dermal absorption and/or of the main urinary metabolites allows taking into account all the factors modulating the extent of exposure, such as the kind of crops, the type of application equipment and the use of protective devices. Other parameters can also be considered, as an example, inhibition of acetylcholinesterase activity could be a biomarker of exposure for widely used organophosphate pesticides with very short half-life (54).

In addition, the complex interaction of host defence mechanisms involved after a genotoxic exposure still need to be understand: interindividual differences in the ability to activate or detoxify genotoxic substances and to repair DNA damage could explain differential susceptibility to pesticides exposure.

The biomonitoring studies including the characterisation of allelic variants for genes involved in the metabolism of xenobiotics (32,33,39,50,53) reported contrasting results. Genetic polymorphisms in paraoxonase genes (*PON*s) were shown to modulate the frequency of MN in subjects exposed to complex mixture of pesticides (53). A recent *in vitro* study (63) showed that paraoxon caused a significant induction of MN only in subjects carrying the *PON1* QQ genotype with a lower PON1 activity, which was not able to hydrolyse the paraoxon.

A final aspect to be pointed out is the use of epithelial cells to evaluate the genetic risk associated to pesticide exposure. It must be emphasised that the MN assay can be applied in interphase to any proliferating cell population and allows the use of epithelial cells. The application of MN assay in buccal or nasal epithelial cells need to be further explored in groups of subjects exposed to pesticides considering the availability of a standardised protocol and of criteria of scoring for MN and other nuclear abnormalities.

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References

1. British Crop Protection Council (2009–2010). *The e-Pesticide Manual*. British Crop Protection Council, Hampshire, UK updated version 5.0.
2. Racke, K. D., Skidmore, M. W., Hamilton, D. J., Unsworth, J. B., Miyamoto, J. and Cohen, S. Z. (1997) Pesticide fate in tropical soils: technical report. *Pure Appl. Chem.*, **69**, 1349–1371.
3. Gonzalez-Arias, C. A. (2010) Patrón de uso y venta de plaguicidas en Nayarit, México. *Rev. Intern. Contam. Amb.*, **3**, 26(in press).
4. Blair, A. and Freeman, L. B. (2009) Epidemiologic studies of cancer in agricultural populations: observations and future directions. *J. Agromedicine*, **14**, 125–131.
5. Hanke, W. and Jurewicz, J. (2004) The risk of adverse reproductive and developmental disorders due to occupational pesticide exposure: an overview of current epidemiological evidence. *Int. J. Occup. Med. Environ. Health*, **17**, 223–243.
6. Lauria, L., Settimi, L., Spinelli, A. and Figà-Talamanca, I. (2006) Exposure to pesticides and time to pregnancy among female greenhouse workers. *Reprod. Toxicol.*, **22**, 425–430.
7. Dhillon, A. S., Tarbutton, G. L., Levin, J. L., Plotkin, G. M., Lowry, L. K., Nalbone, J. T. and Shepherd, S. (2008) Pesticide/environmental exposures and Parkinson's disease in East Texas. *J. Agromedicine*, **13**, 37–48.
8. Costello, S., Cockburn, M., Bronstein, J., Zhang, X. and Ritz, B. (2009) Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of California. *Am. J. Epidemiol.*, **169**, 919–926.
9. Dosunmu, R., Wu, J., Basha, M. R. and Zawia, N. H. (2007) Environmental and dietary risk factors in Alzheimer's disease. *Expert Rev. Neurother.*, **7**, 887–900.
10. Santibáñez, M., Bolumar, F. and García, A. M. (2007) Occupational risk factors in Alzheimer's disease: a review assessing the quality of published epidemiological studies. *Occup. Environ. Med.*, **64**, 723–732.
11. Bolognesi, C. (2003) Genotoxicity of pesticides: a review of human biomonitoring studies. *Mutat. Res.*, **543**, 251–272.
12. Bull, S., Fletcher, K., Boobis, A. R. and Battershill, J. M. (2006) Evidence for genotoxicity of pesticides in pesticide applicators: a review. *Mutagenesis*, **21**, 93–103.
13. da Silva Augusto, L. G., Lieber, S. R., Ruiz, M. A. and de Souza, C. A. (1997) Micronucleus monitoring to assess human occupational exposure to organochlorides. *Environ. Mol. Mutagen.*, **29**, 46–52.
14. Garaj-Vrhovac, V. and Zeljezic, D. (2001) Cytogenetic monitoring of Croatian population occupationally exposed to a complex mixture of pesticides. *Toxicology*, **165**, 153–162.
15. Garaj-Vrhovac, V. and Zeljezic, D. (2002) Assessment of genome damage in a population of Croatian workers employed in pesticide production by chromosomal aberration analysis, micronucleus assay and comet assay. *J. Appl. Toxicol.*, **22**, 249–255.
16. Bhalli, J. A., Khan, Q. M., Haq, M. A., Khalid, A. M. and Nasim, A. (2006) Cytogenetic analysis of Pakistani individuals occupationally exposed to pesticides in a pesticide production industry. *Mutagenesis*, **21**, 143–148.
17. Barbosa, A. and Bonin, M. (1994) Evaluation of phosphine genotoxicity at occupational levels of exposure in New South Wales, Australia. *Occup. Environ. Med.*, **51**, 700–705.
18. Titenko-Holland, N., Windham, G., Kolachana, P., Reinisch, F., Parvatham, S., Osorio, A. M. and Smith, M. T. (1997) Genotoxicity of malathion in human lymphocytes assessed using the micronucleus assay *in vitro* and *in vivo*: a study of malathion-exposed workers. *Mutat. Res.*, **388**, 85–95.
19. Windham, G. C., Titenko-Holland, N., Osorio, A. M., Gettner, S., Reinisch, F., Haas, R. and Smith, M. (1998) Genetic monitoring of malathion-exposed agricultural workers. *Am. J. Ind. Med.*, **33**, 164–174.
20. Calvert, G. M., Talaska, G., Mueller, C. A., Ammenheuser, M. M., Au, W. W., Fajem, J. M., Fleming, L. E., Briggle, T. and Ward, E. (1998) Genotoxicity in workers exposed to methyl bromide. *Mutat. Res.*, **417**, 115–128.
21. Figgs, L. W., Titenko-Holland, N., Rothman, N. *et al.* (2000) Increased lymphocyte replicative index following 2,4-dichlorophenoxyacetic acid herbicide exposure. *Cancer Causes Control*, **11**, 373–380.
22. Vlastos, D., Demsia, G. and Matthopoulos, D. P. (2004) Evaluation of genetic damage in tobacco-growing farmers occupationally exposed to mixtures of metalaxyl and imidacloprid. *Int. J. Env. Anal. Chem.*, **84**, 183–191.
23. Pasquini, R., Scassellati-Sforzolini, G., Angeli, G., Fatigoni, C., Monarca, S., Beneventi, L., DiGiulio, A. M. and Bauleo, F. A. (1996) Cytogenetic biomonitoring of pesticide-exposed farmers in central Italy. *J. Environ. Pathol. Toxicol. Oncol.*, **15**, 29–39.
24. Joksic, G., Vidakovic, A. and Spasojevic-Tisma, V. (1997) Cytogenetic monitoring of pesticide sprayers. *Environ. Res.*, **75**, 113–118.
25. Venegas, W., Zapata, I., Carbonell, E. and Marcos, R. (1998) Micronuclei analysis in lymphocytes of pesticide sprayers from Concepcion, Chile. *Teratog. Carcinog. Mutagen.*, **18**, 123–129.
26. Kehdy, F. S. G., Cerqueira, E. M. M., Bonjardim, M. B., Camelo, R. M. and Castro, M. C. L. (2007) Study of the cytogenetic effects of occupational exposure to pesticides on sanitation workers in Belo Horizonte, Brazil. *Genet. Mol. Res.*, **6**, 581–593.
27. Bolognesi, C., Carrasquilla, G., Volpi, S., Solomon, K. R. and Marshall, E. J. (2009) Biomonitoring of genotoxic risk in agricultural workers from five Colombian regions: association to occupational exposure to glyphosate. *J. Toxicol. Environ. Health A*, **72**, 986–997.
28. Bolognesi, C., Parrini, M., Bonassi, S., Ianello, G. and Salanitto, A. (1993) Cytogenetic analysis of a human population occupationally exposed to pesticides. *Mutat. Res.*, **285**, 239–249.
29. Bolognesi, C., Parrini, M., Reggiardo, G., Merlo, F. and Bonassi, S. (1993) Biomonitoring of workers exposed to pesticides. *Int. Arch. Occup. Environ. Health*, **65** (Suppl. 1), S185–S187.

30. Bolognesi, C., Parrini, M., Merlo, F. and Bonassi, S. (1993) Frequency of micronuclei in lymphocytes from a group of floriculturists exposed to pesticides. *J. Toxicol. Environ. Health*, **40**, 405–411.
31. Scarpato, R., Migliore, L., Angotzi, G., Fedi, A., Miligi, L. and Loprieno, N. (1996) Cytogenetic monitoring of a group of Italian floriculturists: no evidence of DNA damage related to pesticide exposure. *Mutat. Res.*, **367**, 73–82.
32. Scarpato, R., Migliore, L., Hirvonen, A., Falck, G. and Norppa, H. (1996) Cytogenetic monitoring of occupational exposure to pesticides: characterization of GSTM1, GSTT1, and NAT2 genotypes. *Environ. Mol. Mutagen.*, **27**, 263–269.
33. Falck, G. C., Hirvonen, A., Scarpato, R., Saarikoski, S. T., Migliore, L. and Norppa, H. (1999) Micronuclei in blood lymphocytes and genetic polymorphism for *GSTM1, GSTT1* and *NAT2* in pesticide-exposed greenhouse workers. *Mutat. Res.*, **441**, 225–237.
34. Bolognesi, C., Perrone, E. and Landini, E. (2002) Micronucleus monitoring of a floriculturist population from Western Liguria, Italy. *Mutagenesis*, **17**, 391–397.
35. Bolognesi, C., Landini, E., Perrone, E. and Roggieri, P. (2004) Cytogenetic biomonitoring of a floriculturist population in Italy: micronucleus analysis by fluorescence in situ hybridization (FISH) with an all-chromosome centromeric probe. *Mutat. Res.*, **557**, 109–117.
36. Varona, M., Cárdenas, O., Crane, C., Rocha, S., Cuervo, G. and Vargas, J. (2003) Cytogenetic alterations in field workers routinely exposed to pesticides in Bogota flowers farms. *Biomedica*, **23**, 141–152.
37. Bolognesi, C., Merlo, F., Rabboni, R., Roggieri, P., Reggiardo, G. and Abbondandolo, A. (1995) Genotoxic risk from occupational exposure to pesticides in floriculture. *Clin. Chem*, **41**, 1919–1922.
38. Davies, H. W., Kennedy, S. M., Teschke, K., Jenny, P. and Quintana, E. (1998) Cytogenetic analysis of South Asian berry pickers in British Columbia using the micronucleus assay in peripheral lymphocytes. *Mutat. Res.*, **416**, 101–113.
39. Lucero, L., Pastor, S., Suárez, S., Durbán, R., Gómez, C., Parrón, T., Creus, A. and Marcos, R. (2000) Cytogenetic biomonitoring of Spanish greenhouse workers exposed to pesticides: micronuclei analysis in peripheral blood lymphocytes and buccal epithelial cells. *Mutat. Res.*, **464**, 255–262.
40. Ramírez, V. and Cuenca, P. (2001) Micronuclei frequency in lymphocytes of individuals occupationally exposed to pesticides. *Rev. Biol. Trop.*, **49**, 1–8.
41. Pastor, S., Gutiérrez, S., Creus, A., Cebulska-Wasilewska, A. and Marcos, R. (2001) Micronuclei in peripheral blood lymphocytes and buccal epithelial cells of Polish farmers exposed to pesticides. *Mutat. Res.*, **495**, 147–156.
42. Pastor, S., Gutiérrez, S., Creus, A., Xamena, N., Piperakis, S. and Marcos, R. (2001) Cytogenetic analysis of Greek farmers using the micronucleus assay in peripheral lymphocytes and buccal cells. *Mutagenesis*, **16**, 539–545.
43. Pastor, S., Creus, A., Xamena, N., Siffel, C. and Marcos, R. (2002) Occupational exposure to pesticides and cytogenetic damage: results of a Hungarian population study using the micronucleus assay in lymphocytes and buccal cells. *Environ. Mol. Mutagen.*, **40**, 101–109.
44. Pastor, S., Lucero, L., Gutiérrez, S., Durbán, R., Gómez, C., Parrón, T., Creus, A. and Marcos, R. (2002) A follow-up study on micronucleus frequency in Spanish agricultural workers exposed to pesticides. *Mutagenesis*, **17**, 79–82.
45. Pastor, S., Creus, A., Parrón, T., Cebulska-Wasilewska, A., Siffel, C., Piperakis, S. and Marcos, R. (2003) Biomonitoring of four European populations occupationally exposed to pesticides: use of micronuclei as biomarkers. *Mutagenesis*, **18**, 249–258.
46. Márquez, C., Villalobos, C., Poblete, S., Villalobos, E., García, M. A. and Duk, S. (2005) Cytogenetic damage in female Chilean agricultural workers exposed to mixtures of pesticides. *Environ. Mol. Mutagen.*, **45**, 1–7.
47. Vlastos, D., Stivaktakis, P. and Matthopoulos, D. P. (2006) Pesticide exposure and genotoxicity correlations within a Greek farmers' group. *Int. J. Env. Anal. Chem.*, **86**, 215–223.
48. Tope, A., Bebe, F. N. and Panemangalore, M. (2006) Micronuclei frequency in lymphocytes and antioxidants in the blood of traditional limited-resource farm workers exposed to pesticides. *J. Environ. Sci. Health B*, **41**, 843–853.
49. Montero, R., Serrano, L., Araujo, A., Dávila, V., Ponce, J., Camacho, R., Morales, E. and Méndez, A. (2006) Increased cytogenetic damage in a zone in transition from agricultural to industrial use: comprehensive analysis of the micronucleus test in peripheral blood lymphocytes. *Mutagenesis*, **21**, 335–342.
50. Costa, C., Teixeira, J. P., Silva, S. *et al.* (2006) Cytogenetic and molecular biomonitoring of a Portuguese population exposed to pesticides. *Mutagenesis*, **21**, 343–350.
51. Costa, C., Silva, S., Coelho, P., Roma-Torres, J., Teixeira, J. P. and Mayan, O. (2007) Micronucleus analysis in a Portuguese population exposed to pesticides. Preliminary survey. *Int. J. Hyg. Environ. Health*, **210**, 415–418.
52. Ali, T., Bhalli, J. A., Rana, S. M. and Khan, Q. M. (2008) Cytogenetic damage in female Pakistani agricultural workers exposed to pesticides. *Environ. Mol. Mutagen.*, **49**, 374–380.
53. da Silva, J., Moraes, C. R., Vanina, D., Heuser, V., Andrade, M. and Silva, F. R. (2008) Evaluation of genetic damage in a Brazilian population occupationally exposed to pesticides and its correlation with polymorphisms in metabolizing genes. *Mutagenesis*, **23**, 415–422.
54. Levario-Carillo, M., Sordo, M., Rocha, F., González-Horta, C., Amato, D. and Ostrosky-Wegman, P. (2005) Micronucleus frequency in human umbilical cord lymphocytes. *Mutat. Res.*, **586**, 68–75.
55. Remor, A. P., Totti, A. C., Moreira, D. A., Dutra, G. P., Heuser, V. D. and Boeira, J. M. (2009) Occupational exposure of farm workers to pesticides: biochemical parameters and evaluation of genotoxicity. *Environ. Int.*, **35**, 273 278.
56. Gómez-Arroyo, S., Díaz-Sánchez, Y., Meneses-Pérez, M. A., Villalobos-Pietrini, R. and De León-Rodríguez, J. (2000) Cytogenetic biomonitoring in a Mexican floriculture worker group exposed to pesticides. *Mutat. Res.*, **466**, 117–124.
57. Ergene, S., Celik, A., Cavas, T. and Kaya, F. (2007) Genotoxic biomonitoring study of population residing in pesticide contaminated regions in Goksu delta: micronucleus, chromosomal aberrations and sister chromatid exchanges. *Environ. Int.*, **33**, 877–885.
58. Martínez-Valenzuela, C., Gómez-Arroyo, S., Villalobos-Pietrini, R., Waliszewski, S., Calderón-Segura, M. E., Félix-Gastélum, R. and Alvarez-Torres, A. (2009) Genotoxic biomonitoring of agricultural workers exposed to pesticides in the north of Sinaloa State, Mexico. *Environ. Int.*, **35**, 1155–1159.
59. Sailaja, N., Chandrasekhar, M., Rekhadevi, P. V., Mahboob, M., Rahman, M. F., Vuyyuri, S. B., Danadevi, K., Hussain, S. A. and Grover, P. (2006) Genotoxic evaluation of workers employed in pesticide production. *Mutat. Res.*, **609**, 74–80.
60. Bortoli, G. M., Azevedo, M. B. and Silva, L. B. (2009) Cytogenetic biomonitoring of Brazilian workers exposed to pesticides: micronucleus analysis in buccal epithelial cells of soybean growers. *Mutat. Res.*, **675**, 1–4.
61. Castro, R., Ramírez, V. and Cuenca, P. (2004) Micronuclei and other nuclear abnormalities in the oral epithelium of female workers exposed to pesticides. *Rev. Biol. Trop.*, **52**, 611–621.
62. Fenech, M. and Morley, A. (1985) Solutions to the kinetic problem in the micronucleus assay. *Cytobios*, **43**, 233–246.
63. Rojas-Garcia, A. E., Sordo, M., Vega, L., Quintanilla-Vega, B., Solis-Heredia, M. and Ostrosky-Wegman, P. (2009) The role of paraoxonase polymorphisms in the induction of micronucleus in paraoxon-treated human lymphocytes. *Environ. Mol. Mutag.*, **50**, 823–829.

Differences in the carcinogenic evaluation of glyphosate between the International Agency for Research on Cancer (IARC) and the European Food Safety Authority (EFSA)

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The International Agency for Research on Cancer (IARC) Monographs Programme identifies chemicals, drugs, mixtures, occupational exposures, lifestyles and personal habits, and physical and biological agents that cause cancer in humans and has evaluated about 1000 agents since 1971. Monographs are written by ad hoc Working Groups (WGs) of international scientific experts over a period of about 12 months ending in an eight-day meeting. The WG evaluates all of the publicly available scientific information on each substance and, through a transparent and rigorous process,[1] decides on the degree to which the scientific evidence supports that substance's potential to cause or not cause cancer in humans.

For Monograph 112,[2] 17 expert scientists evaluated the carcinogenic hazard for four insecticides and the herbicide glyphosate.[3] The WG concluded that the data for glyphosate meet the criteria for classification as a *probable human carcinogen*.

The European Food Safety Authority (EFSA) is the primary agency of the European Union for risk assessments regarding food safety. In October 2015, EFSA reported[4] on their evaluation of the Renewal Assessment Report[5] (RAR) for glyphosate that was prepared by the Rapporteur Member State, the German Federal Institute for Risk Assessment (BfR). EFSA concluded that 'glyphosate is unlikely to pose a carcinogenic hazard to humans and the evidence does not support classification with regard to its carcinogenic potential'. Addendum 1 (the BfR Addendum) of the RAR[5] discusses the scientific rationale for differing from the IARC WG conclusion.

Serious flaws in the scientific evaluation in the RAR incorrectly characterise the potential for a carcinogenic hazard from exposure to glyphosate. Since the RAR is the basis for the European Food Safety Agency (EFSA) conclusion,[4] it is critical that these shortcomings are corrected.

THE HUMAN EVIDENCE

EFSA concluded 'that there is very limited evidence for an association between glyphosate-based formulations and non-Hodgkin lymphoma (NHL), overall inconclusive for a causal or clear associative relationship between glyphosate and cancer in human studies'. The BfR Addendum (p. ii) to the EFSA report explains that 'no consistent positive association was observed' and 'the most powerful study showed no effect'. The IARC WG concluded there is *limited evidence of carcinogenicity in humans* which means "A positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence."[1]

The finding of *limited evidence* by the IARC WG was for NHL, based on high-quality case–control studies, which are particularly valuable for determining the carcinogenicity of an agent because their design facilitates exposure assessment and reduces the potential for certain biases. The Agricultural Health Study[6] (AHS) was the only cohort study available providing information on the carcinogenicity

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of glyphosate. The study had a null finding for NHL (RR 1.1, 0.7–1.9) with no apparent exposure–response relationship in the results. Despite potential advantages of cohort versus case–control studies, the AHS had only 92 NHL cases in the unadjusted analysis as compared to 650 cases in a pooled case–control analysis from the USA.[7] In addition, the median follow-up time in the AHS was 6.7 years, which is unlikely to be long enough to account for cancer latency.[8]

The RAR classified all of the case–control studies as 'not reliable,' because, for example, information on glyphosate exposure, smoking status and/or previous diseases had not been assessed. In most cases, this is contrary to what is actually described in the publications. Well-designed case–control studies are recognised as strong evidence and routinely relied on for hazard evaluations.[9 10] The IARC WG carefully and thoroughly evaluated all available epidemiology data, considering the strengths and weaknesses of each study. This is key to determining that the positive associations seen in the case–control studies are a reliable indication of an association and not simply due to chance or methodological flaws. To provide a reasonable interpretation of the findings, an evaluation needs to properly weight studies according to quality rather than simply count the number of positives and negatives. The two meta-analyses cited in the IARC Monograph[11] are excellent examples of objective evaluations and show a consistent positive association between glyphosate and NHL.

The final conclusion[5] (Addendum 1, p.21) that "there was no unequivocal evidence for a clear and strong association of NHL with glyphosate" is misleading. IARC, like many other groups, uses three levels of evidence for human cancer data.[1] *Sufficient evidence* means 'that a causal relationship has been established' between glyphosate and NHL. BfR's conclusion is equivalent to deciding that there is not *sufficient evidence*. Legitimate public health concerns arise when 'causality is credible', that is, when there is *limited evidence of carcinogenicity.*

EVIDENCE FROM ANIMAL CARCINOGENICITY STUDIES

EFSA concluded 'No evidence of carcinogenicity was confirmed by the majority of the experts (with the exception of one minority view) in either rats or mice due to a lack of statistical significance in pairwise comparison tests, lack of consistency in multiple animal studies and slightly increased incidences only at dose levels at or above the limit dose/maximum tolerated dose (MTD), lack of preneoplastic lesions and/or being within historical control range'. The IARC WG review found a significant positive trend for renal tumours in male CD-1 mice,[12] a rare tumour, although no comparisons of any individual exposure group to the control group were statistically significant. The WG also identified a significant positive trend for hemangiosarcoma in male CD-1 mice,[13] again with no individual exposure group significantly different from controls. Finally, the WG also saw a significant increase in the incidence of pancreatic islet cell adenomas in two studies in male Sprague-Dawley rats.[14–16] In one of these rat studies, thyroid gland adenomas in females and liver adenomas in males were also increased. By the IARC review criteria,[1] this constitutes *sufficient evidence* in animals.

The IARC WG reached this conclusion using data that were publicly available in sufficient detail for independent scientific evaluation (a requirement of the IARC Preamble[1]). On the basis of the BfR Addendum, it seems there were three additional mouse studies and two additional rat studies that were unpublished and available to EFSA. Two of the additional studies were reported to have a significant trend for renal tumours, one in CD-1 mice (Sugimoto. *18-Month Oral Oncogenicity Study in Mice*. Unpublished, designated ASB2012–11493 in RAR. 1997), and one in Swiss-Webster mice (Unknown. *A chronic feeding study of glyphosate (roundup technical) in mice*. Unpublished, designated ABS2012–11491 in RAR. 2001). One of these studies (Sugimoto. Unpublished, 1997) also reported a significant trend for hemangiosarcoma. The RAR also reported two studies in CD-1 mice showing significant trends for malignant lymphoma (Sugimoto. Unpublished, 1997; Unknown. *Glyphosate Technical: Dietary Carcinogencity Study in the Mouse*. Unpublished, designated ABS2012–11492 in RAR. 2009).

The RAR dismissed the observed trends in tumour incidence because there are no individual treatment groups that are significantly different from controls and because the maximum observed response is reportedly within the range of the historical control data (Table 5.3–1, p.90). Care must be taken in using historical control data to evaluate animal carcinogenicity data. In virtually all guidelines,[1 17 18] scientific reports[19] and publications[20–23] on this issue, the recommended first choice is the use of concurrent controls and trend tests, even in the EC regulations cited in the RAR[18] (see p.375). Trend tests are more powerful than pairwise comparisons, particularly for rare tumours where data are sparse. Historical control data should be from studies in the same time frame, for the same animal strain, preferably from the same laboratory or the same supplier and preferably reviewed by the same pathologist.[17 18] While the EFSA final peer review[4] mentions the use of historical control data from the original laboratory, no specifics are provided and the only referenced historical control data[24] are in the BfR addendum.[5] One of the mouse studies[12] was clearly done before this historical control database was developed, one study (Sugimoto. Unpublished, 1997) used Crj:CD-1 mice rather than Crl:CD-1 mice, and one study[13] did not specify the substrain and was reported in 1993 (probably started prior to 1988). Hence, only a single study (Unknown. Unpublished, 2009) used the same mouse strain as the cited historical controls, but was reported more than 10 years after the historical control data set was developed.

The RAR dismissed the slightly increased tumour incidences in the studies considered because they occurred "only at dose levels at or above the limit dose/maximum tolerated dose (MTD)", and because there was a lack of preneoplastic lesions. Exceeding the MTD is demonstrated by an increase in mortality or other serious toxicological findings at the highest dose, not by a slight reduction in body weight. No serious toxicological findings were reported at the highest doses for the mouse studies in the RAR. While some would argue that these high doses could cause cellular disruption (eg, regenerative hyperplasia) leading to cancer, no evidence of this was reported in any study. Finally, a lack of preneoplastic lesions for a significant neoplastic finding is insufficient reason to discard the finding.

MECHANISTIC INFORMATION

The BfR Addendum dismisses the IARC WG finding that 'there is strong evidence that glyphosate causes genotoxicity' by suggesting that unpublished evidence not seen by the IARC WG was overwhelmingly negative and that, since the reviewed studies were not done under guideline principles, they should get less weight. To maintain transparency, IARC reviews only publicly available data. The use of confidential data submitted to the BfR makes it impossible for any scientist not associated with BfR to review this conclusion. Further weakening their interpretation,

the BfR did not include evidence of chromosomal damage from exposed humans or human cells that were highlighted in Tables 4.1 and 4.2 of the IARC Monograph [3]

The BfR confirms (p.79) that the studies evaluated by the IARC WG on oxidative stress were predominantly positive but does not agree that this is strong support for an oxidative stress mechanism. They minimise the significance of these findings predominantly because of a lack of positive controls in some studies and because many of the studies used glyphosate formulations and not pure glyphosate. In contrast, the WG concluded that (p.77) 'Strong evidence exists that glyphosate, AMPA and glyphosate-based formulations can induce oxidative stress'. From a scientific perspective, these types of mechanistic studies play a key role in distinguishing between the effects of mixtures, pure substances and metabolites.

Finally, we strongly disagree that data from studies published in the peer-reviewed literature should automatically receive less weight than guideline studies. Compliance with guidelines and Good Laboratory Practice does not guarantee validity and relevance of the study design, statistical rigour and attention to sources of bias.[25 26] The majority of research after the initial marketing approval, including epidemiology studies, will be conducted in research laboratories using various models to address specific issues related to toxicity, often with no testing guidelines available. Peer-reviewed and published findings have great value in understanding mechanisms of carcinogenicity and should be given appropriate weight in an evaluation based on study quality, not just on compliance with guideline rules.

GENERAL COMMENTS

Science moves forward on careful evaluations of data and a rigorous review of findings, interpretations and conclusions. An important aspect of this process is transparency and the ability to question or debate the findings of others. This ensures the validity of the results and provides a strong basis for decisions. Many of the elements of transparency do not exist for the RAR.[5] For example, citations for almost all references, even those from the open scientific literature, have been redacted. The ability to objectively evaluate the findings of a scientific report requires a complete list of cited supporting evidence. As another example, there are no authors or contributors listed for either document, a requirement for publication in virtually all scientific journals where financial support, conflicts of interest and affiliations of authors are fully disclosed. This is in direct contrast to the IARC WG evaluation listing all authors, all publications and public disclosure of pertinent conflicts of interest prior to the WG meeting.[27]

Several guidelines have been devised for conducting careful evaluation and analysis of carcinogenicity data, most after consultation with scientists from around the world. Two of the most widely used guidelines in Europe are the OECD guidance on the conduct and design of chronic toxicity and carcinogenicity studies[17] and the European Chemicals Agency Guidance on Commission Regulation (EU) No 286/2011;[18] both are cited in the RAR. The methods used for historical controls and trend analysis are inconsistent with these guidelines.

Owing to the potential public health impact of glyphosate, which is an extensively used pesticide, it is essential that all scientific evidence relating to its possible carcinogenicity is publicly accessible and reviewed transparently in accordance with established scientific criteria.

SUMMARY

The IARC WG concluded that glyphosate is a 'probable human carcinogen', putting it into IARC category 2A due to *sufficient evidence* of carcinogenicity in animals, *limited evidence* of carcinogenicity in humans and *strong* evidence for two carcinogenic mechanisms.

- The IARC WG found an association between NHL and glyphosate based on the available human evidence.
- The IARC WG found significant carcinogenic effects in laboratory animals for rare kidney tumours and hemangiosarcoma in two mouse studies and benign tumours in two rat studies.
- The IARC WG concluded that there was strong evidence of genotoxicity and oxidative stress for glyphosate, entirely from publicly available research, including findings of DNA damage in the peripheral blood of exposed humans.

The RAR concluded[5] (Vol. 1, p.160) that 'classification and labelling for carcinogenesis is not warranted' and 'glyphosate is devoid of genotoxic potential'.

- EFSA[4] classified the human evidence as 'very limited' and then dismissed any association of glyphosate with cancer without clear explanation or justification.
- Ignoring established guidelines cited in their report, EFSA dismissed evidence of renal tumours in three mouse studies, hemangiosarcoma in two mouse studies and malignant lymphoma in two mouse studies. Thus, EFSA incorrectly discarded all findings of glyphosate-induced cancer in animals as chance occurrences.
- EFSA ignored important laboratory and human mechanistic evidence of genotoxicity.
- EFSA confirmed that glyphosate induces oxidative stress but then, having dismissed all other findings of possible carcinogenicity, dismissed this finding on the grounds that oxidative stress alone is not sufficient for carcinogen labelling.

The most appropriate and scientifically based evaluation of the cancers reported in humans and laboratory animals as well as supportive mechanistic data is that glyphosate is a *probable human carcinogen*. On the basis of this conclusion and in the absence of evidence to the contrary, it is reasonable to conclude that glyphosate formulations should also be considered likely human carcinogens. The CLP Criteria[18] (Table 3.6.1, p.371) allow for a similar classification of Category 1B when there are 'studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals'.

In the RAR, almost no weight is given to studies from the published literature and there is an over-reliance on non-publicly available industry-provided studies using a limited set of assays that define the minimum data necessary for the marketing of a pesticide. The IARC WG evaluation of *probably carcinogenic to humans* accurately reflects the results of published scientific literature on glyphosate and, on the face of it, unpublished studies to which EFSA refers.

Most of the authors of this commentary previously expressed their concerns to EFSA and others regarding their review of glyphosate[28] to which EFSA has published a reply.[29] This commentary responds to the EFSA reply.

The views expressed in this editorial are the opinion of the authors and do not imply an endorsement or support for these opinions by any organisations to which they are affiliated.

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Competing interests CJP, MTS and DDW are providing advice to a US law firm involved in glyphosate litigation. CJP also works part-time for the Environmental Defense Fund on issues not related to pesticides.

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REFERENCES

1 IARC. *Preamble to the IARC Monographs*. 2006. http://monographs.iarc.fr/ENG/Preamble/CurrentPreamble.pdf
2 Guyton KZ, Loomis D, Grosse Y, *et al*. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. *Lancet Oncol* 2015;16:490–1.
3 IARC Working Group. Glyphosate. In: *Some organophosphate insecticides and herbicides: diazinon, glyphosate, malathion, parathion, and tetrachlorvinphos*. Vol 112. IARC Monogr Prog, 2015:1–92.
4 European Food Safety Authority. Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. *EFSA J* 2015;13:4302.
5 European Food Safety Authority. *Final Addendum to the Renewal Assessment Report*. 2015. http://registerofquestions.efsa.europa.eu/roqFrontend/outputLoader?output=ON-4302
6 De Roos AJ, Blair A, Rusiecki JA, *et al*. Cancer incidence among glyphosate-exposed pesticide applicators in the Agricultural Health Study. *Environ Health Perspect* 2005;113:49–54.
7 De Roos AJ, Zahm SH, Cantor KP, *et al*. Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. *Occup Environ Med* 2003;60:E11.
8 Weisenburger DD. Pathological classification of non-Hodgkin's lymphoma for epidemiological studies. *Cancer Res* 1992;52(19 Suppl):5456s–62s; discussion 5462s-5464s.
9 Checkoway H, Pearce N, Kriebel D. Research methods in occupational epidemiology. 2nd edn. *Monographs in epidemiology and biostatistics*. New York: Oxford University Press, 2004:xiv, 372 p.
10 Rothman KJ, Greenland S, Lash TL. *Modern epidemiology*. 3rd edn. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins, 2008:x, 758 p.
11 Schinasi L, Leon ME. Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: a systematic review and meta-analysis. *Int J Environ Res Public Health* 2014;11:4449–527.
12 EPA. *Glyphosate; EPA Reg. # 524–308; mouse oncogenicity study*. 1985. V. Accession No. 251007–014. Tox review 004370. p.
13 JMPR. FAO plant production and protection paper, 178, 2004—pesticide residues in food—2004. *Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and WHO the Core Assessment Group*; Vol 178. Geneva, Switzerland: World Health Organization/Food and Agriculture Organization, 2004:95–169.
14 EPA. *Second peer review of Glyphosate*. 19911–19.
15 EPA. *Glyphosate—EPA Registration No. 524–308–2-Year Chronic Feeding/Oncogenicity Study in Rats with Technical Glyphosate*, I. William Dykstra. Toxicology Branch, Editor. 1991. V. MRID 416438–01 Tox review 008897. p.
16 EPA. *Glyphosate; 2-Year Combined Chronic Toxicity/Carcinogenicity Study in Sprague-Dawley Rats—List A Pesticide for Reregistration*, B. William Dykstra. Toxicology, Editor. 1991. V. MRID 416438–01. Tox review 008390. 1–29.
17 OECD. *Guidance Document 116 on the Conduct and Design of Chronic Toxicity and Carcinogenicity Studies*, H.a.S.P. Environment, Editor. Paris: OECD, 2012.
18 European Chemicals Agency. *Guidance on the Application of the CLP Criteria: Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures*. Helsinki, Finland: European Chemicals Agency, 2015.
19 NRC Committee to Review the Styrene Assessment in The National Toxicology Program 12th Report on Carcinogens. *Review of the Styrene Assessment in the National Toxicology Program 12th Report on Carcinogens: Workshop Summary*; Washington DC: National Academies Press, 2014.
20 Keenan C, Elmore S, Francke-Carroll S, *et al*. Best practices for use of historical control data of proliferative rodent lesions. *Toxicol Pathol* 2009;37:679–93.
21 Haseman JK, Boorman GA, Huff J. Value of historical control data and other issues related to the evaluation of long-term rodent carcinogenicity studies. *Toxicol Pathol* 1997;25:524–7.
22 Greim H, Gelbke HP, Reuter U, *et al*. Evaluation of historical control data in carcinogenicity studies. *Hum Exp Toxicol* 2003;22:541–9.
23 Haseman JK, Huff J, Boorman GA. Use of historical control data in carcinogenicity studies in rodents. *Toxicol Pathol* 1984;12:126–35.
24 Giknis M, Clifford C. *Spontaneous Neoplastic Lesions in the Crl:CD-1(ICR)BR Mouse*. Charles River Laboratories; 2000.
25 Myers JP, vom Saal FS, Akingbemi BT, *et al*. Why public health agencies cannot depend on good laboratory practices as a criterion for selecting data: the case of bisphenol A. *Environ Health Perspect* 2009;117:309–15.
26 Buonsante VA, Muilerman H, Santos T, *et al*. Risk assessment's insensitive toxicity testing may cause it to fail. *Environ Res* 2014;135:139–47.
27 IARC Monograph 112. *List of Working Group Participants*. IARC Monogr Eval Carcinog Risks Hum 2015 26 March, 2015 [cited 2015 24 November]. http://monographs.iarc.fr/ENG/Meetings/vol112-participants.pdf
28 Portier CJ, *et al*. *Open Letter: Review of the Carcinogenicity of Glyphosate by EFSA and BfR*. 2015 [cited 2016 1/18/2016]. http://www.efsa.europa.eu/sites/default/files/Prof_Portier_letter.pdf
29 Url B. Response to Open Letter: Review of the Carcinogenicity of Glyphosate by EFSA and BfR. 2016. 1/13/2016. http://www.efsa.europa.eu/sites/default/files/EFSA_response_Prof_Portier.pdf