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Summary Histopathology Data for 18 Month Control Study
Safepharm Laboratories Internal Project 0041-0216

The attached document was prepared to summarize the histopathology of neoplasms observed in an internal project (number 0041-0216) conducted by Safepharm Laboratories Ltd.

Safepharm Laboratories Ltd. was acquired initially by Harlan Laboratories Ltd. and subsequently Huntingdon Life Sciences. The company now operates as part of Envigo.

I can confirm that I was the Study Director for the project (number 0041-0216) and was responsible for the presentation of this summary.

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Observations on the Development of Spontaneous Neoplasms in Male and in Female Crl: CD-1 (ICR) BR Strain Mice Following 18-Months on Control Diet.

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Safepharm Laboratories Ltd, PO Box 45, Derby DE1 2BT
(Internal Publication)

SUMMARY

Male and female Crl: CD-1 (ICR) BR strain mice were maintained for a period of 18 months on standard laboratory diet at the Safepharm Laboratories Limited animal facility. Food and water were provided *ad libitum*. All mice found dead or killed *in extremis* during the course of the investigation together with those killed at termination were subjected to a full necropsy examination and histopathological evaluation. All neoplastic lesions were identified and a tabulation of spontaneous tumour incidences compiled.

METHODS

50 male and 50 female mice of the Crl: CD-1 (ICR) BR strain were supplied by Charles River (UK) Limited, Margate, Kent. Mice were aged 6-8 weeks at the start of the study and the weight variation did not exceed ± 20% of the mean weight for either sex. Animals were maintained at 21 ± 2° C with a target humidity of 55 ±15%. Lighting was 12 hours of continuous artificial light in each 24 hour period and there were at least 15 air changes per hour. Mice were housed in groups of no more than 3 by sex in polypropylene cages with wood flake bedding.

Rodent 5LF2 (Certified) diet (BCM IPS Limited, London, UK) with batch analysis, and tap water were provided *ad libitum*, and both the diet and the drinking water were routinely analysed to ensure the absence of any contaminant that could reasonably be anticipated to influence tumour development. Animals were provided with wooden chew blocks and cardboard tunnels both of which were routinely sampled and analysed for any contaminants having the potential to influence the purpose or integrity of the investigation. The study was conducted according to Safepharm policy on animal welfare and to the requirements of the United Kingdom's Animals (Scientific Procedures) Act 1986 in order to cause the minimum of suffering or distress to animals.

Mice were inspected twice daily, early and late during the working periods, for mortality or signs of ill-health and clinical signs were observed daily throughout the duration of the study. Detailed clinical examination was performed once a week and the findings recorded. From week 48 onward animals were palpated and the location, size and description of any masses recorded. Bodyweights were recorded on Day 1 of the study, at four-weekly intervals thereafter, and at termination.

A blood smear was prepared for all animals during the final week of the study (Week 78) and differential white cell counts performed. Post mortem studies were undertaken on all animal found dead or killed *in extremis* and on those killed at termination of the study. Mice killed *in extremis* and those killed at termination were euthanased by exsanguination following intravenous overdose of sodium pentobarbitone. A full external and internal necropsy examination was performed on all animals and a comprehensive list of organs and tissues taken or sampled into 10% neutral buffered formalin.

Tissue were routinely processed to paraffin wax, sectioned, and stained with haematoxylin and eosin. All tissues were examined microscopically and all neoplastic lesions reported. Non-neoplastic conditions were observed but not reported since they were not the primary objective of this investigation.

RESULTS

This investigation is concerned only with the development of neoplastic lesions. Non-neoplastic conditions were observed in most organs and tissues and these were entirely consistent with age-related degenerative or atrophic changes seen commonly in ageing or geriatric mice. Secondary changes were also seen in mice that developed severely debilitating neoplastic pathology. No unexpected non-neoplastic changes were encountered.

Of the 50 male and 50 female mice examined none was lost to autolysis or cannibalism. A total of 51 tumours was seen, 23 of these occurring in male mice and 28 in females. 12 (24%) male mice and 12 (24%) female mice died before scheduled termination. 20 (40%) male mice and 21 (42%) female mice had at least one neoplasm and 3 (6%) male mice and 6 (12%) female mice developed multiple neoplasms at different sites. 6 premature death male mice and 8 premature death female had tumours. 6 (12%) male mice and 6 (12%) female mice developed malignant lymphoma and one (2%) female mouse was diagnosed with myeloid leukaemia. Unusually, histiocytic sarcoma was not reported for any female mice in this study although this tumour type is relatively commonly seen in females, particularly in the uterus, of this mouse strain. No testicular or ovarian tumours were observed.

A breakdown of tumour incidence by type including all tumours encountered in the study is given in Table 1.

SUMMARY AND CONCLUSION:

Care needs to be exercised when interpreting the incidences of neoplastic lesions from mouse oncogenicity studies employing group size of 50 per sex because appreciable differences can be encountered in the prevalence of commonly reported tumours, even though a group size of 50 animals per sex is considered to provide a strong statistical basis for evaluation. The absence of a particular tumour type in control mice can suggest an effect of treatment when that tumour is present among high dose animals. However, the 'effect' in such cases can be a consequence of an absence among control animals and not a presence among treated mice. The compilation of background data on the prevalence of neoplastic conditions among control mice is thus of great value in assessing the true incidence of conditions, but the incidences between batches of animals, and thus the variability of distribution, is of as much, of not more, value than the overall background prevalence.

This investigation provided incidences of neoplastic lesions that developed in male and in female mice maintained on a standard rodent diet during a period of 18 months. Although the group size was relatively small many of the tumour types commonly encountered in laboratory mice were observed and these can be compared with incidences from other batches of mice maintained on the same diet for the same duration at the Safepharm animal facility.

Table 1

Incidence and % Incidence of Neoplastic Lesions by Tissue for Terminal Kill and Interim Death Animals Combined

Number of Mice	Males 50		Females 50	
CONDITION	**n**	**%**	**n**	**%**
ADRENAL GLAND				
Cortical adenoma b	4	8	0	0
BONE				
Osteoma b	0		1	2
HARDERIAN GLAND				
Adenoma b	4	8	2	4
GASTRO-INTESTINAL TUMOUR				
Stomach adenocarcinoma m	1	2	0	0
LIVER				
Hepatocellular carcinoma m	3	6	0	0
Haemangiosarcoma m	1	2	0	0
LUNG				
Adenoma b	0	0	1	2
Adenocarcinoma m	2	4	1	2
Combined	2	4	2	4
MAMMARY GLAND				
Adenocarcinoma m	0	0	3	6
Adenosquamous carcinoma m	0	0	1	2
PITUITARY				
Adenoma b	0	0	1	2
SKIN/SUBCUTIS				
Fibrosarcoma m	1	2	0	0
OVARY				
Luteoma b			1	2
UTERUS				
Endometrial stromal polyp b			4	8
Leiomyoma b			3	6
Stromal sarcoma m			2	4
Haemangiosarcoma m			1	2
THYROID				
Adenoma b	1	2	0	0
LYMPHOID/HAEMOPOIETIC				
Myeloid leukaemia m	0	0	1	2
Malignant lymphoma m	6	12	6	12
*Histiocytic sarcoma m	0	0	0	0
Combined	6	12	7	14
OVERALL TUMOUR INCIDENCE				
Primary benign tumours	9	18	10	20
Primary malignant tumours	14	28	14	28
Multiple benign tumours	0	0	0	0
Multiple malignant tumours	1	2	2	4
Multiple any tumours	3	6	4	8

* Histiocytic sarcomas are not generally regarded as lymphoid in origin but are usefully included here