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Atomic Energy Control Board
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RBE OF TRITIUM BETA RAYS FOR CAUSES OF DEATH OTHER THAN MYELO LEUKEMIA IN MALE CBA/H MICE

(AECB Project No. 2.154.3)

by

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Research report

RBE OF TRITIUM BETA RAYS FOR CAUSES OF DEATH OTHER THAN MYELOID LEUKEMIA IN MALE CBA/H MICE

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ABSTRACT

Causes of death were examined for 5,206 male CBA/H mice which had previously been treated with tritiated water or with X rays at comparable doses and comparable dose rates. Data on induced myeloid leukemia had been examined in detail in a previous report. The purpose of the present study was to examine the relative biological effectiveness of tritium beta rays for causes of death other than mye-loid leukemia. However, no consistent values for the tritium relative biological effectiveness were obtained. The values were spread over a wide range for different endpoints and were generally less reliable than those for induction of myeloid leukemia. A surprising decrease in time to death of animals without tumours was observed in the irradiated groups of mice. This observation suggests that a detailed review of recent data on non-specific life shortening in irradiated animals and humans might be useful.

RÉSUMÉ

Le présent rapport fait état des résultats de l'étude sur les causes de mortalité de 5206 souris CBA/H mâles qui avaient été préalablement traitées avec de l'eau tritiée ou des rayons X à des doses et à des débits de dose comparables. Les données sur les cas de leucémie myéloïde provoqués ont déjà été analysées dans un rapport antérieur, tandis que le présent rapport vise à estimer l'efficacité biologique relative des rayons bêta du tritium comme autre cause de mortalité que la leucémie myéloïde. On n'a pourtant obtenu aucune valeur cohérente de l'efficacité biologique relative du tritium. Les valeurs s'étalaient sur une grande échelle et étaient généralement moins fiables que celles obtenues dans les cas de leucémie myéloïde provoqués. On a cependant remarqué que les animaux sans tumeur parmi les groupes irradiés de souris mouraient plus rapidement. Cette observation pourrait laisser entendre qu'il serait utile de procéder à un examen détaillé des données récentes sur la réduction non spécifique de la vie chez les animaux et les humains irradiés.

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A. INTRODUCTION

In order to help resolve uncertainties as to the most appropriate quality factor for tritium beta rays, a large experiment was carried out earlier to measure the relative biological effectiveness (RBE) of tritiated water compared to X-rays for the induction of myeloid leukemia (ML) in male mice of the CBA/H strain [1]. A total of 279 cases of ML were diagnosed among the 5,206 mice used in this experiment and the RBE of tritium for induction of ML was estimated to be close to 1.2 [1]. However, there was also a decrease in average age at death of the 4,181 irradiated mice which did not die of ML. Since a decrease in average life expectancy caused by low doses of radiation at low dose rate is normally ascribed to radiation-induced cancers [2], it was considered useful to examine the other causes of death for these mice in more detail.

The purpose of the present study was to estimate the RBE of tritium beta rays for causes of death other than ML in the previous study [1] of male CBA/H mice that had been treated with comparable doses of tritiated water and of X-rays at comparable dose rates. The results of this analysis, which will be presented below, did not provide any consistent RBE values.

B. METHODS

The basic design of the study and the treatment of the mice is described in detail in previous reports [1,3,4]. In brief, male mice were exposed at about 99 days of age to single injections of tritiated water (HTO) or to X-rays at comparable and decreasing dose rates over a period of 10 days. The control group contained 747 animals while the six irradiated groups contained between 732 and 754 animals each (Table 1). Total radiation doses in the tritium groups designated as HTO-1, HTO-2 and HTO-3 were estimated to be 0.85, 1.86 and 3.04 Gy respectively, while the X-ray groups designated as X-1, X-2 and X-3 received 1.06, 1.98 and 2.64 Gy respectively [1].

As noted in the previous report [1], blood samples from 10 of our mice were tested in 1986 by the Institut Armand-Frappier, Laval, Quebec, and reported to be free of infection by pathogenic viruses or bacteria. The method used to test for pathogenic viruses was complement fixation. A new and different group of CBA mice from Harwell was tested in 1990 using the more sensitive ELISA method and was reported to contain low titres of MHV hepatitis virus and of GD7 encephalomyelitis virus. In retrospect, it seems probable that the same two viruses may have been present in our mice in 1986 but at titres so low as to escape detection by the complement fixation method. However, in the opinion of the pathologist (D.H. Percy) and veterinarian (H.M. Wyatt) involved in this study, the presence of these viruses did not affect the death of the mice used in this long-term study.

In some cases, as will be noted later, deaths were associated with diseases typical of bacterial infection, e.g., bronchopneumonia, but not with diseases typical of hepatitis or encephalomyelitis virus.

Following the radiation treatments, the mice were held under standard conditions in the animal facility at the Chalk River laboratories and were checked six days per week for the remainder of their life. Mice were housed with an average of 7 animals per cage, 35 cages per rack. Each cage was tagged with information identifying cage number, set, treatment, date of birth and number of animals alive. The racks were filled in a consecutive manner so as to maximize available space. Therefore more than one group of a single treatment or a group of a different treatment could be housed on the same rack. The racks were then randomly placed in one of three housing rooms in the animal facility. When an animal was found dead or euthanized, the date and state of the animal (ie dead or euthanized) was recorded on the cage tag. Examination of the animals was performed on a room rotation basis to minimize bias. Each animal was held, palpated if required and examined for appearance, mobility, general behaviour and colour of feet. On occasion, a mouse had to be separated due to cage-mates fighting, or an individual sick animal was separated, and housed in a clear polycarbonate shoebox for closer observation. These shoebox cages were placed on top of the rack in which they originated. Animals which were found dead in their cage and animals which appeared to be moribund or suffering were subject to postmortem examination. Blood samples were drawn at the time of necropsy on all euthanized animals and analyzed for total white blood cell (WBC) count and hemoglobin level. A blood smear was prepared and analyzed for a WBC differential and red blood cell morphology. All internal organs in the abdomen and thorax were examined (see details in Appendix A) and any indications of pathological changes in these or other organs (for example ear or the eye) were noted. For each case, tissue samples were taken from the liver, kidneys(2), adrenals(2), spleen, the thoracic unit, sternum and lower vertebrae plus any other tissue in which abnormalities were observed at postmortem. The gross pathology was noted on a primary file. The tissues were prepared for microscopic analysis by fixation in 10% buffered neutral formalin; hard tissues were decalcified; all tissues were then processed for embedding in paraffin wax, cut and stained with Hematoxylin and Eosin. The histopathology slide preparations along with the respective data sheets identifying the condition of the animal and the gross pathology report with a tentative diagnosis from the primary report were sent to the Ontario Veterinary College at the University of Guelph and examined for diagnosis by an experienced pathologist. Examples of the data sheets and primary files are illustrated in Appendix B.

The diagnoses and pathological findings for each tissue were entered in coded form into a personal computer, using the standard codes that are illustrated in Appendix C. These coded diagnoses, along with other relevant information such as date of birth, date of the beginning of the radiation treatment and date of death of each animal, were then used for sorting and statistical analyses on the main-frame computer at the Chalk River laboratories. The results of these analyses are presented below.

C. RESULTS

Table 1 shows the number of mice in each group with and without tumors. The fraction of mice with ML increased from 0.1% to about 6% in the irradiated groups as noted in the previous report [1]. The fraction of mice with tumors other than ML increased from 53% in the control group to an average of about 61% in the irradiated groups, while the fraction dying without tumors decreased correspondingly from 47% in the control group to an average of about 33% in the irradiated groups.

As noted previously, the average age at death was decreased by 50.7 days in the three groups treated with X-rays and by 40.7 days in the three groups treated with HTO [1]. The tritium RBE suggested by these values would be about 0.8. About 86% of this total decrease in the irradiated groups was due to causes other than the induction of ML [1]. Since this average is based on deaths occuring at various times between 400 and 1000 days of age [see Fig. 1], the standard deviations on average age at death were large and usually in the region of + 40 days for any given group. The standard deviations on the decrease in average age at death were correspondingly large and thus slightly greater than the actual decrease in average age at death for any of the irradiated groups. More precise values based on time to death of 50% of the mice in each group were therefore examined in the present report. Two methods were used, the first being the day at which 50% of the animals had died as derived from a simple plot of cumulative mortality against time [see Fig. 1] (Table 2), while the second was based on a linear regression analysis of the data points between cumulative mortalities of 35% and 65% (Table 3). In general, the latter data provided a good fit to a straight line, the correlation coefficients for goodness of fit varying between 0.991 and 1.000 with values of 0.997 or 0.998 for the majority of the data. All of the numbers in Tables 2 and 3 are precise values with potential errors of less than one day, since they are based on the exact date of the start of treatment and on exact dates of death to within 0-1 day. Since the data in Table 2 are observed and not calculated values, no standard deviations can be given. The standard deviations on the time to death of 50% of the mice in each group as determined by linear regression analysis (Table 3) were small, as might be expected from the high correlation coefficients noted above. The ranges of standard deviations on time in days from start of treatment to death of 50% of the mice in each of the seven groups were as follows for the categories listed in Table 3:

Other tumors	0.7 - 1.2 days	(1.5 days)
Tissue abnormality	1.6 - 3.6 days	(4.1 days)
No abnormality	1.6 - 4.0 days	(4.6 days)
Total without tumor	1.1 - 2.8 days	(3.2 days)
Total except ML	0.3 - 1.9 days	(1.9 days)
Total	0.7 - 1.6 days	(1.9 days)

The data in brackets given on the previous page represent the average of six values for the standard deviation of the decrease in time to death in the irradiated groups for each of the categories listed in Table 3. With one exception (no tissue abnormality or tumor, group HTO-1, decrease 3 + 5.3 days), the decrease in time to death in each of the irradiated groups for all categories listed in Table 3 was statistically significant at the 95% confidence level. Potential sources of bias in determination of the time of death, usually by euthanasia, will be considered later in this report.

Three points were noted with both methods of analysis (Tables 2 and 3):
(a) The time to 50% cumulative mortality was 30-40 days longer for animals dying without a tumor than for animals dying with a tumor.
(b) The radiation treatments decreased the time to 50% mortality for animals dying without a tumor as well as for animals dying with a tumor.
(c) The decrease in time to 50% cumulative mortality was greater for animals treated with X-rays than for animals treated with HTO.

Table 4 summarizes the incidence of various general categories of tumors. Further details on types of tumors found in the liver and lung are given in Table 5. It should be noted that these data are expressed as total tumors in each group, not as number of animals with a tumor as in Table 1. More than one tumor was found in some animals, usually a tumor of the liver plus a lung tumor. The average number of tumors per animal with a tumor was 1.20 (see Totals in Tables 1 and 4).

The data on ML were analysed in detail in the previous report [1]. Apart from ML, consistent trends towards an increase in tumor incidence in the irradiated groups was observed only for liver tumors (Table 4), notably liver carcinomas and hepatomas (Table 5), and lung adenomas (Table 5). A small increase in incidence of lymphomas was observed in the irradiated groups but the increase was of borderline significance. No consistent and significant increases were observed for the other categories of tumors examined.

Data on average time from the start of treatment to death associated with each category of tumor were also routinely available from the computer-assisted analysis of the death records for each of the mice. Representative values are given in Table 6. Because the numbers of animals in each category (Tables 4 and 5) were relatively small, the data are less reliable than those based on total animals with tumors (Tables 2 and 3). In general, cases of ML appeared much earlier than did other tumors in the irradiated groups. No further analysis of these data was carried out.

Some 63% of the animals without tumors did show other pathological abnormalities at postmortem (Table 1). Typical abnormalities included non-malignant hyperplastic nodules, peri-hepatitis and biliary cysts in the liver, bronchopneumonia, mononuclear infiltration and focal alveolitis in the lung, torticollis of the ear, and cystic tubules, focal tubular degeneration, hydronephrosis and nephritis in the kidney. Data on the incidence of these abnormalities in animals without tumors are shown in Table 7. No consistent increases in the incidence of these abnormalities were observed in the irradiated groups.

Blood cell counts and differential white blood cell analyses were available for about 90% of the mice [1]. Examination of these data showed zero cases of myeloid or lymphoid hyperplasia that were not associated either with a tumor or with other non-tumor tissue abnormalities. All cases of increased white blood cell counts could therefore be attributed to pathological changes in other tissues.

Tritium RBE values were estimated for those endpoints which exhibited consistent changes between the control and irradiated groups (Table 8). The slopes "b" of the individual regression lines and the standard deviation (S.D.) of the resulting RBE values were calculated in the same manner as described in the previous report [1] (see Appendix D). For those values where the S.D. of the calculated RBE did not exceed the RBE itself, the RBE values ranged from 0.53 + 0.16 to 3.46 + 3.17. The RBE values for the increase in incidence of various tumors (excluding ML) in irradiated animals were usually greater than one but did not differ significantly from 1.0, assuming 95% confidence intervals equal to twice the S.D. The RBE values for radiation-induced decrease in time to 50% death of the animals were consistently less than one (Table 8) and in a few cases (for example, 0.53 + 0.16 for decrease in time to 50% death of animals with tumors) were significantly less than 1.0.

As a rough correction for possible cell killing at high radiation doses, the analyses were repeated using only the first 3 data points in each sequence, omitting the highest radiation dose, as well as with all 4 data points. In some cases this change reduced and in other cases increased the S.D. of the RBE (Table 8). It did not seem profitable to pursue this approach in more detail. The observed radiation-induced changes were too small and the quality of much of the resulting data was not good enough to yield consistent tritium RBE values.

The latter conclusion does not apply to the original data on ML, where the radiation treatments induced marked changes in incidence [1]. Analysis of the ML data (Tables 1 and 4) using the simple equation $I = bD$ as in Table 8B yielded tritium RBE values of 1.12 + 0.26 when all 4 data points in each sequence were used [1] and of 1.18 + 0.28 when only the first 3 data points (omitting the highest dose) in each sequence were used.

D. DISCUSSION

The results of this analysis were rather disappointing in as far as they failed to provide any consistent RBE values for tritium beta rays for endpoints other than induction of ML. Further consideration of the data has not provided any reason to alter the original conclusion [1] that the tritium RBE for induction of ML was in the region of 1.2 + 0.3. Calculated tritium RBE values for other selected endpoints in this experiment were less precise and varied from about 0.5 to 3.5. The only RBE values that were significantly different from 1.0 were smaller than 1.0.

It is of some interest to compare the results of the present study on male CBA/H mice with those given in previous publications from other laboratories. It is well known that a large variability exists between different strains of mice with respect to the incidence of different types of tumor [cf.2]. The CBA colony at Harwell had reportedly been selectively bred for longevity [5]. The average life-span of the control group of male CBA/H mice used in the present study was 767 days [1], which is slightly greater than the value of 685 days at Harwell [5] or than the value of 710 days cited for the same strain at laboratories near Rome [6]. However, the average tumor incidence appeared to be appreciably lower for the group of animals used in the present study at Chalk River than for those studied earlier at Harwell (Table 9). This difference may be due to genetic drift, since it is known that the characteristics of a mouse colony which is not inbred may shift over the course of time due to random selection of different genetic characteristics in the parents of each generation. Under these circumstances, some of the minor differences in results obtained at the two laboratories would not appear to be serious; for example, the Harwell study failed to find any consistent increase in liver tumor incidence in irradiated animals, which is not too surprising in view of the high spontaneous incidence of liver tumors, together with the failure to analyse for specific types of tumor, in the Harwell study [5]. Even an apparent decrease in incidence of certain tumors (e.g. reticulum cell sarcoma) which have a high natural incidence in certain strains of mice is not uncommon at high radiation doses [9].

A more serious concern is the observed decrease in time to death of animals without tumors (Tables 2 and 3). The topic of non-specific life shortening due to radiation exposure (i.e, life shortening due to causes other than radiation-induced tumors) was last reviewed in detail in the 1982 UNSCEAR report [2]. It was agreed at that time that non-specific life shortening was evident in some animals that survived exposure to radiation at high doses and high dose rate, sufficient to approach doses which would cause early death due to bone marrow or gastrointestinal failure. However, there was no reliable evidence of similar effects for low doses of radiation at low dose rates.

To quote from paragraph 216 on page 688 of this report [2]: "If non-specific life shortening is viewed as an advancement in time of diseases normally associated with senescence without apparent changes in the spectrum of these diseases, no data are found to support such a concept... Particularly at the low doses and dose rates of interest in radiation protection there appears to be no need to invoke any general non-specific noxious effect, because all the experience on animals does not require to postulate any other effect than tumour induction or acceleration to explain the reduced life expectancy observed after irradiation." Similar conclusions have been suggested in earlier reports from other scientific committees.

The 1982 UNSCEAR report [2] did not assign a high priority to further research on this topic. One of the most recent research reports by Thomson and Grahn [7] on life shortening in mice exposed to continuous gamma radiation at low dose rate notes that the data on days of life lost per unit radiation dose "were not significantly altered when the analysis was restricted to those mice dying with tumors (92% of the total) or to those presumably dying from tumors (82% of the total). Analysis of mortality rates showed that about 90% of the radiation-specific excess mortality was tumor related." In view of the high rates of spontaneous tumor incidence in the cited study, these data [7] do not provide strong evidence against non-specific life shortening by radiation exposure at low dose rate.

The observed decrease in time to death in animals dying without tumors in the present study (Tables 2 and 3) is somewhat surprising in view of the 1982 UNSCEAR conclusions [2] cited above. Sources of possible bias resulting from the experimental procedures used to produce the present data have been considered. The control group and all of the six irradiated groups were each further subdivided into six subgroups for which treatments were commenced at different times over a period of 4.5 months [1]; these six subgroups of each of the seven treatment groups were scattered at random in three animal holding rooms at the Chalk River animal facility. The majority of animals did not die a natural death; in fact, about 92% of the animals were euthanized because they appeared to be moribund or suffering[1]. There was thus considerable subjective judgement in the selection of mice for euthanasia each day, these judgments being based on individual interpretations of the recommendations of the Chalk River Animal Care Committee (see Appendix E) and thus on those of the supervisory body, the Canadian Council on Animal Care [8]. A total of four persons were involved in the daily examinations and postmortems of the mice in any given week, and these persons were rotated from one room to another on a regular basis (Appendix F). It is thus difficult to locate any particular source of bias which would affect the average age at death of the mice in a given group.

Another potential source of bias might be the dose rate. Because it was necessary to inject three times as much tritium initially to obtain three times the total of the lowest dose, dose rates were linked to total dose and vary by three-fold for a three-fold variation in total dose of tritium beta-rays. X-ray dose rates were similarly varied by three-fold in order to achieve comparable dose rates for both radiation sources [1]. The average dose rates in the present study varied from about 0.085 to 0.3 Gy per day or 0.06 to 0.21 milliGy per minute [1]. These dose rates can be considered to be relatively low by UNSCEAR standards, which define low dose rates of low LET radiation as less than 0.05 milliGy per minute [9], while high dose rates are defined as greater than 50 milliGy per minute [9]. This definition appears to be based primarily on studies on induction of somatic mutations in spiderwort, of chromosomal aberrations in human lymphocytes and of mammary tumors in female rats. For induction of coat colour mutations in mouse spermatogonia, on the other hand, low dose rates of gamma-radiation appear to extend from 0.007 up to about 8 milliGy per minute [10]. The 1990 draft recommendations of the ICRP define low dose rates as less than 0.1 Gy per hour or 1.6 milliGy per minute of low LET radiation. All of the dose rates used in the present study would be low by this ICRP definition. The three-fold variation in dose rate used in the present study is expected to have little effect upon the RBE values, particularly since all of the dose rates could be categorised as near the low dose rate region. A further decrease in dose rates should have little effect on the observed RBE values for tritium beta-rays, while a 1000-fold increase in dose rate might be expected to decrease the RBE values for induction of ML from about 1.2 to approach closer to 1.0. This conclusion, which should apply not only to induction of ML [1] but also to induction of chromosomal aberrations in lymphocytes and of translocation in spermatogonia of CBA/H mice [11], is based on analogy with the observed larger decrease in RBEs for fast neutrons from values of 10 to 50 at low doses and low dose rates to values of 2 to 3 at high doses and high dose rates [2,9].

The reasons for the observed decrease in time to death of animals without tumors in the irradiated groups of mice were not apparent. The present data are, in our opinion, too limited to provide a sufficient basis to refute the conclusions of the 1982 UNSCEAR report [2] on the absence of non-specific life shortening as a result of exposure to low doses of radiation at low dose rate.

However, these data might be judged sufficient to justify a critical detailed review of the research data on this topic, particularly of those data which have been published since 1982. (Further reason for this review might possibly be provided by some of the more extreme but not necessarily scientific statements on this topic which have been provided to the Canadian public [12]). As recommended in the 1982 UNSCEAR report [2],

highest research priority should be given to the effects of radiation in causing or contributing to various causes of death in humans, notably in the atomic bomb survivors in Japan. Since it is anticipated that further data on causes of death other than cancer may soon become available for the Hiroshima - Nagasaki survivors, a detailed review of relevant data derived both from animal and human studies might be appropriate when these latter data become available.

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TABLE 1. Number of mice with tumors and tissue abnormalities in each group

Category	Group				% of Total Mice		
	Control	X-1 HTO-1	X-2 HTO-2	X-3 HTO-3	Control	All X	All HTO
ML	1	34 43	48 50	45 60	0.13 +0.13)	5.7 (+0.5)	6.8 (+0.6)
Other tumors (excluding ML)	398	420 431	414 481	442 512	53.2 (+2.7)	57.5 (+1.6)	63.6 (+1.7)
Tissue abnormalities but no tumors	215	183 162	171 146	171 112	28.8 (+2.0)	23.7 (+1.0)	18.8 (+0.9)
No tissue abnormalities or tumors	133	97 96	106 77	88 70	17.8 (+1.5)	13.1 (+0.7)	10.8 (+0.7)
Total without tumors	348	280 258	277 223	259 182	46.6 (+2.5)	36.8 (+1.3)	29.6 (+1.1)
Total	747	734 732	739 754	746 754	100.	100.	100.

TABLE 2. Time in days from start of treatment to death of 50% of mice in each group, as determined directly from a plot of cumulative mortality against time

Category	Group				Decrease (in days)	
	Control	X-1 HTO-1	X-2 HTO-2	X-3 HTO-3	Control minus all x	Control minus all HTO
Other tumors (except ML)	658	602 630	613 633	607 625	51	29
Tissue abnormality but no tumor	729	655 689	652 656	655 680	75	54
No tissue abnormality or tumor	664	639 672	642 647	630 613	27	20
Total with no tumors	704	649 683	648 653	646 654	56	41
Total except ML	673	613 649	627 640	621 633	53	32
Total (including ML)	673	612 644	620 632	612 625	58	39

TABLE 3. Time in days from start of treatment to death of 50% of mice in each group, as determined by a linear regression analysis on data from 35 to 65% of deaths

Category	Group				Decrease (in days)	
	Control	X-1 HTO-1	X-2 HTO-2	X-3 HTO-3	Control minus all X	Control minus all HTO
Other tumors (except ML)	662	603 632	612 635	605 626	55	31
Tissue abnormality but no tumor	716	649 685	653 653	663 677	61	44
No tissue abnormality or tumor	668	633 665	640 649	635 613	32	26
Total with no tumors	698	644 678	648 652	653 652	50	37
Total except ML	678	619 648	627 640	620 632	56	38
Total (including ML)	678	616 643	619 634	613 626	62	44

TABLE 4. Total number of tumors in each group

Category	Group				Incidence of tumors per 100 animals		
	Control	X-1 HTO-1	X-2 HTO-2	X-3 HTO-3	Control	All X	All HTO
ML	1	34 43	48 50	45 60	0.13 (+0.13)	5.7 (+0.5)	6.8 (+0.6)
Liver (except hemangiosarcoma)	214	274 290	259 310	303 324	28.6 (+2.0)	37.7 (+1.3)	41.3 (+1.4)
Lung	122	118 105	109 147	113 152	16.3 (+1.5)	15.3 (+0.8)	18.0 (+0.9)
Harderian gland (eye)	40	34 39	35 38	31 46	5.4 (+0.8)	4.5 (+0.5)	5.5 (+0.5)
Hemangio -sarcomas*	38	33 32	36 24	42 40	5.1 (+0.8)	5.0 (+0.5)	4.3 (+0.4)
Lymphoma	16	20 19	19 27	17 41	2.1 (+0.5)	2.5 (+0.3)	3.9 (+0.4)
Reticulum cell sarcoma**	18	20 18	19 23	12 41	2.4 (+0.6)	2.3 (+0.3)	3.7 (+0.4)
Kidney	2	1 1	3 6	5 6	0.3 (+0.2)	0.4 (+0.1)	0.6 (+0.2)
All other	18	8 11	10 12	11 16	2.4 (+0.6)	1.3 (+0.2)	1.7 (+0.3)
Total except ML	468	509 515	490 587	534 666	62.6 (+2.9)	69.1 (+1.8)	78.9 (+1.9)
Total (including ML)	469	543 558	538 637	579 726	62.8 (+2.9)	74.8 (+1.8)	85.7 (+2.0)

(* Note 1: most hemangiosarcomas were located either in the liver or in the spleen. Hemangiosarcomas in the liver have been separated out in Table 5.)

(** Note 2: evidence of reticulum cell sarcoma was frequently found in multiple organs in the same animal, for example, in bone marrow, liver, lung and spleen. No attempt was therefore made to classify reticulum cell sarcoms by the organ of origin.)

TABLE 5. Numbers of liver and lung tumors of different types in each group

Type of tumor	Group				Incidence of tumors per 100 animals		
	Control	X-1 HTO-1	X-2 HTO-2	X-3 HTO-3	Control	All X	All HTO
Liver carcinoma	84	93 103	94 107	113 124	11.2 (+1.2)	13.5 (+0.8)	14.9 (+0.8)
Liver hepatoma	126	177 179	163 199	184 190	16.9 (+1.5)	23.6 (+1.0)	25.4 (+1.1)
Liver hemangiosarcoma	25	21 17	24 14	25 22	3.3 (+0.7)	3.2 (+0.4)	2.4 (+0.3)
Other liver tumor	4	4 3	2 4	6 10	0.5 (+0.3)	0.5 (±0.2)	0.8 (±0.2)
Lung carcinoma	93	77 68	64 100	83 102	12.4 (+1.3)	10.1 (±0.7)	12.1 (±0.7)
Lung adenoma	27	38 37	41 44	29 44	3.6 (+0.7)	4.9 (±0.5)	5.6 (±0.5)
Other lung tumor	2	3 0	4 3	1 6	0.3 (±0.2)	0.4 (±0.1)	0.4 (±0.1)

TABLE 6. Average time in days from start of treatment to deaths associated with various categories of tumor

Category of tumor	Control	Group X-1 HTO-1	Group X-2 HTO-2	Group X-3 HTO-3	Average All X	Average All HTO
ML	-	539 563	460 512	510 521	503	532
Liver carcinoma	592	551 577	549 595	579 598	560	590
Liver hepatoma	625	594 623	609 622	593 618	599	621
Lung carcinoma	745	700 684	657 678	645 673	667	678
Lung adenoma	624	641 639	639 655	621 572	628	622
Harderian gland	758	674 686	719 725	682 707	692	706
Hemangio -sarcomas	732	664 702	619 682	633 676	639	687
Lymphoma	699	709 618	686 562	604 518	666	566

TABLE 7. Total number of tissue abnormalities in each group of mice without any tumor

Tissue	Group			Incidence of abnormalities per 100 animals without tumors		
	Control	X-1 HTO-1	X-2 HTO-2	X-3 HTO-3	Control	All X	All HTO
Liver	72	93 72	80 49	75 38	18.6 (±2.2)	27.8 (±1.8)	19.0 (±1.5)
Lung	124	104 87	82 73	93 60	32.0 (±2.9)	31.2 (±1.9)	26.3 (±1.8)
Kidney	29	18 27	33 43	16 21	7.5 (±1.4)	7.5 (±0.9)	10.9 (±1.1)
Other	29	13 16	16 11	16 13	7.5 (±1.4)	5.0 (±0.8)	4.8 (±0.8)
Total	254	228 202	211 176	200 132	65.6 (±4.1)	71.6 (±2.8)	61.1 (±2.7)

TABLE 8. RBE of tritium compared to X-rays for selected endpoints

(A) Data fitted to the equation I = a + bD, where I is incidence of tumors per 100 mice and D is dose in Gy

Number of data points used	Radiation source	a + S.D.	b + S.D.	RBE + S.D.
All liver tumors except hemangiosarcomas (Table 4):				
4	HTO	32.0 + 3.3	4.22 + 1.78	
	X	30.0 + 2.7	3.82 + 1.56	1.11 + 0.65
3	HTO	30.6 + 3.9	6.54 + 3.27	
	X	30.3 + 4.0	3.36 + 3.06	(1.95 + 2.02)*
Liver carcinoma (Table 5):				
4	HTO	11.8 + 0.7	1.54 + 0.38	
	X	11.1 + 0.7	1.29 + 0.41	1.19 + 0.48
3	HTO	11.8 + 1.1	1.54 + 0.92	
	X	11.4 + 0.5	0.76 + 0.37	2.02 + 1.55
Liver hepatoma (Table 5):				
4	HTO	19.6 + 2.8	2.51 + 1.51	
	X	18.4 + 2.3	2.49 + 1.31	1.01 + 0.81
3	HTO	18.0 + 2.4	5.02 + 2.00	
	X	18.2 + 3.4	2.73 + 2.60	(1.84 + 1.90)*
Lung adenoma (Table 5)				
4	HTO	4.06 + 0.48	0.71 + 0.26	
	X	4.25 + 0.98	0.22 + 0.57	(3.26 + 8.52)*
3	HTO	3.76 + 0.31	1.19 + 0.26	
	X	3.77 + 0.40	0.99 + 0.31	1.20 + 0.46
Lymphoma (Table 4):				
4	HTO	1.87 + 0.30	1.09 + 0.16	
	X	2.35 + 0.28	0.05 + 0.16	(21.2 + 65.8)*
3	HTO	2.07 + 0.14	0.78 + 0.12	
	X	2.25 + 0.26	0.23 + 0.20	3.46 + 3.17

* RBE values are given in brackets because the standard deviation on the RBE is greater than the RBE itself.

TABLE 8 (Continued)

(B) Data fitted to the equation I = bD, where I is the decrease in time to 50% death of the mice and D is dose in Gv.

Number of data points used	Radiation source	b + S.D.	RBE + S.D.
All animals with tumors except ML (Table 3)			
4	HTO	13.8 + 3.0	
	X	26.0 + 5.6	0.53 + 0.16
[Table 2]			[0.54 + 0.17]
3	HTO	18.1 + 5.6	
	X	32.0 + 8.9	0.57 + 0.23
[Table 2]			[0.57 + 0.25]
All animals without tumors (Table 3)			
4	HTO	18.1 + 2.5	
	X	22.9 + 5.6	0.79 + 0.22
[Table 2]			[0.74 ± 0.17]
3	HTO	24.5 + 0.3	
	X	31.0 + 7.6	0.79 + 0.19
[Table 2]			[0.80 ± 0.17]
Total animals except ML (Table 3)			
4	HTO	17.6 ± 2.8	
	X	26.4 ± 5.5	0.67 ± 0.18
[Table 2]			[0.62 + 0.18]
3	HTO	23.0 + 4.0	
	X	32.4 + 8.8	0.71 ± 0.23
[Table 2]			[0.64 + 0.22]

TABLE 9. Lifetime tumor incidence in untreated male CBA/H mice

Category of tumor	Tumor incidence per 100 mice		
	Present Study	Major 1979 [5]	Di Majo 1986 [6]
ML	0.13	0.0	0.0
Liver*	32.0	72.6	-
Lung	16.3	26.3	-
Harderian gland	5.4	12.6	17.
Reticulum cell sarcoma	2.4	2.1	-
All other	9.9	6.9	-

(* Includes hemangiosarcomas in the liver.)

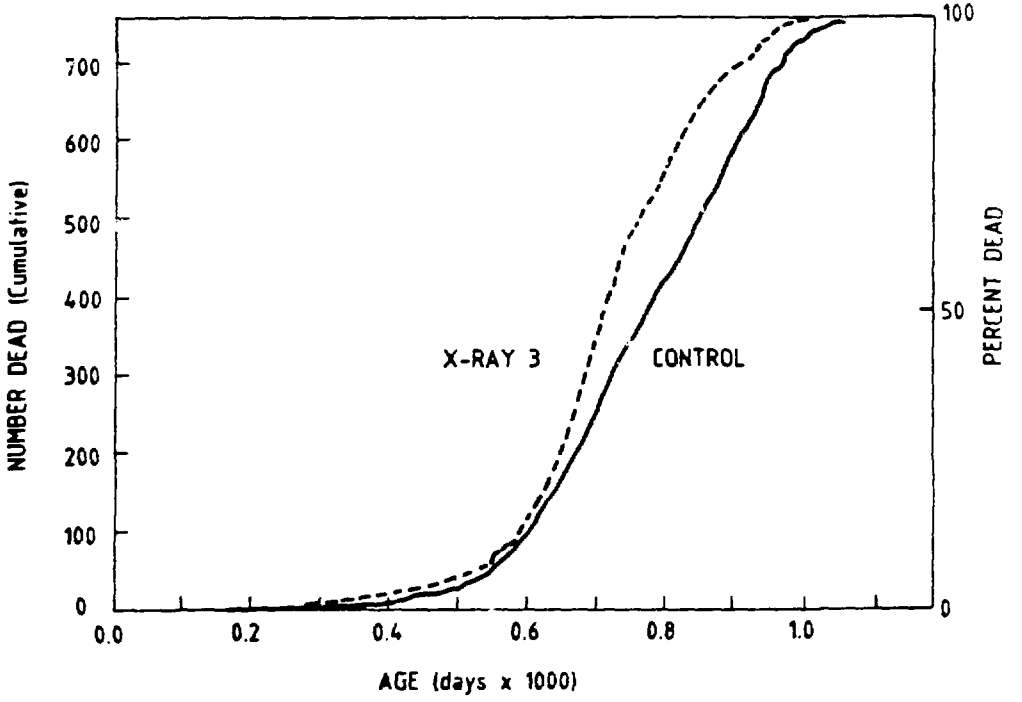

Figure 1. Cumulative mortality of all mice in the control group and in the group designated X-ray 3. Cumulative mortality curves for other irradiated groups are not shown but were similar to that for group X-3.

APPENDIX A

POSTMORTEM PROCEDURES

Procedures for Checking and Sampling

1. Colony should be checked daily, using the "inventory method", i.e. animals in each cage must be counted and the number cross-checked for accuracy against the cage tag.

2. If an animal does not look healthy, it should be put separate in a shoebox cage for observation, having appropriately tagged both cage of origin and the shoebox cage. The animal must be weighed daily and the weights recorded.

3. If the animal is moribund and/or suffering, it should be euthanized by decapitation, at which time blood is collected for WBC, RBC and a blood smear. Other tissues are collected as per Pathology Form, indicated by an asterisk. Additional tissues and samples must be taken of organs that look grossly abnormal. A medical history (as much as possible) and any other gross observations (e.g. anemia, jaundice, texture and colour of organs, etc.) should be recorded and may prove very useful in diagnosis.

4. If an animal is found dead in its cage, then describe where and under what circumstances the animal is found. Record when death in your opinion took place. Always collect tissues as per Pathology Form, irregardless of time of death. After collection of tissues, put animal in a platic bag with the same number as the Sample number and put it in the freezer. In this way we can have another look at the carcass.

5. Tissue collection procedure:

   (a) Preparation:
   - sample bottle filled with formalin (10% buffered neutral formalin from Fisher Scientific)
   - fill in "History and Miscellaneous Information" on the pathology form
   - record animal's date of death and sample number on the cage card
   - have ready a "mini-blood collection vial".

   (b) Carcass condition - record how long in your opinion this animal has been dead (if found dead in cage).

   (c) If animal was alive, then animal should be anesthetized (bell jar with methoxyflurane) and decapitated, followed by immediate blood collection in the "mini-blood collection vials". Make sure to "flick" the vial gently with your finger during collection, and also to keep the collection time to less than 30 seconds. These precautions are essential so that no clots form in the blood which will dramatically change the WBC and the RBC. If a clot can be seen then record this.

(d) Take the blood down to the hospital and using the Coulter Counter obtain a value for WBC and RBC. Enter these values on the Pathology record. Also mark a slide with the sample number and do a bloodsmear which is air dried immediately and must be stored in the box marked "blood" in the fridge of the Animal Facility.

(e) Open the skin on the ventral (lower) side of the animal across the length of the animal. Look for any abnormalities in the subcutaneous tissues including the lymph nodes under the front and hind legs. If these lymph nodes are large, then take one sample.

(f) Open the abdominal musculature across the entire abdomen, and cut through the ribs on one side of and close to the sternum all the way along the sternum. Now cut along the other side of the sternum and remove it from the animal. Trim off excess muscle and lay it on a hard surface. Using a scalpel blade "slit" across each segment of the sternum so that the formalin can reach the marrow. Drop the sternum in the sample bottle.

(g) Look for any gross abnormalities in any of the internal tissues and take samples as appropriate, recording any particulars on the Pathology Form.

(h) In the absence of any gross abnormalities take a small sample of the liver, both kidneys and the whole of both adrenal glands. The spleen should be removed and cut transverse in the middle.

(i) At this point the "pluck" should be removed. This is the combination of the heart, lungs, thymus, which are all attached to one another at the mediastinum, through which the esophagus goes. To remove the pluck, dissect away the tissues which attach it to the back of the animal, starting at the lungs, lifting it up as you go and continuing towards the head. Once the lung and heart are loose from the back of the animal, you can cut through the esophagus and take it out of the animal. With a 1 cc syringe and 27 gauge needle, inject some formalin into each lobe of the lung and into the heart. Then drop the whole pluck into the sample bottle.

(j) Also we would like to collect approximatley two vertebrae. To do this use a bone rongeurs and sever the spinal cord in two places which are about 2-3 vertebrae apart. Lift the segment out, trim off the excess muscle and use a scalpel blade to slit the vertebrae so that the formalin will be able to fix the marrow.

ANIMAL IDENTIFICATION

Sample No:	HISTORY and MISCELLANEOUS INFORMATION
Treatment:	
DOB:	
Set:	
Cage:	
Animal:	
Disposition: D=dead, E=euth, A=acc. m=morib, S=suffering	
Date:	

GROSS FINDINGS Necropsy by:_____ Histology ___ taken
___ missing

Carcass Condition	*	
Blood	*	Smear:_____
Lym. Nd (Cerv.) (Inguin.) (Axill.)(Mesent.)		
Marrow (Sternum) (vertebrae)	* *	
Pituitary		
Lung	*	
Heart		
Thymus	*	
Liver	*	
Gall Bladder		
Spleen	*	Smear _____
Kidney	*	
Adrenal Gland	*	
Gastrointestinal		
Bladder		
Anus		
Testis/Ovary		
Sem.Ves./Uterus		
Ext. Genitalia		
Skin, Mouth/Lips/Eyes		

APPENDIX B

EXAMPLES OF THE DIAGNOSTIC RECORDS PREPARED FOR EACH OF THE 5,206 MICE

ANIMAL IDENTIFICATION

B-1

Sample No: 78	HISTORY and MISCELLANEOUS INFORMATION
Treatment: HTO-2	
DOB: 86 7 26	
Set: 2	
Cage: 17	anemic, hunched?
Animal: 3	skin and mouth
Disposition: D=dead, E=euth, A=acc. m=morib, S=suffering	
Date: 88 3 14	

GROSS FINDINGS Necropsy by: O Histology ___ taken ___ missing

Carcass Condition	*	
Blood	✓	WBC= 11.600 RBC= 4.84m Smear:
Lym. Nd (Cerv.) (Inguin.) (Axill) (Mesent.)		1x2 ? small - (mammary cyst fluid)
Marrow (Sternum)	*	pale
(vertebrae)	* ✓	
Pituitary		
Lung	* ✓	pale, enlarged
Heart		
Thymus	*	
Liver	* ✓	enlarged, tan
Gall Bladder		
Spleen	* ✓	grossly enlarged, red irregular stipple - diff infil
Kidney	* ✓	pale
Adrenal Glands (2)	* ✓	pale
Gastrointestinal		
Bladder		
Anus		
Testis/Ovary		
Uterus/Uterine		

SAMPLE #: 578	SET	TREATMENT	CAGE	MOUSE	ML	CAUSE-OF-DEATH
	02	HTO2	17	3	F	14Re

B M L L S K A 1 2 3
KnPnPnEnErPaP Lr

DOB: 86.07.26 DOT: 86.10.27 DOD: 88.03.14

ANEMIC, ERECT, CRUSTED PENIS. WBC:11600 RBC:4.84M LYMPH NODES.-1-SMALL? MAMMOR CYST AXILL. 2- 1*2CM MESENT. LUNGS PALE, ENLARGED. LIVER ENLARGED, TAN. SPLEEN GROSSLY ENLAGD, RED IRREGULAR SHAPE, DIFF. INFILTRATED. KIDNEY PALE. AD GL PAL

blood smear, bone marrow, liver, lung- nvl kidney- mineralized casts spleen, pancreatic lymph node- diffuse infiltation with primitive reticulum type cells

dx: rcs

SAMPLE #: 578	SET	TREATMENT	CAGE	MOUSE	ML	CAUSE-OF-DEATH
	02	HTDI	17	3	F	13RE

DOB: 86.07.26 DOT: 86.10.27 DOD: 88.03.14
AGE (DAYS): 596

ANEMIC, ERECT, CRUSTED PENIS. WBC:11600 RBC:4.84M LYMPH NODES.-1-SMALL? MAMMORY CYST AXILL. 2- 1*2CM MESENT. LUNGS PALE, ENLARGED. LIVER ENLARGED, TAN. SPLEEN GROSSLY ENLAGD. RED IRREGULAR SHAPE, DIFF. INFILTRATED. KIDNEY PALE. AD GL PALE. DX; RCS

Kidney - Mineralized casts / calculi present in scattered tubules

Spleen, pancreatic Lymph Node - Diffuse infiltration with primitive reticulum type cells-

Bone Marrow
Liver, Lung } NVL

D+ Reticulum Cell Sarcoma

PATHOLOGY RECORD

ANIMAL IDENTIFICATION

Sample No: ~~[illegible]~~ 601	HISTORY and MISCELLANEOUS INFORMATION
Treatment: X-3	
DOB: 86.10.18	[illegible]
Sex: ♀	[illegible]
Cage: 11	
Animal: 1	
Disposition: D=dead, E=euth, A=acc. M=morib, S=suffering	
Date: 88 3 18	

GROSS FINDINGS Necropsy by: [illegible] Histology ___ taken ___ missing

Carcass Condition	*	[illegible]
Blood	* ✓	WBC= 6.800 RBC= ~~[illegible]~~ 1.98M Smear:___
Lymph Nd (Cerv.) (Inguin.) (Axill.)(Mesent.)		
Marrow (Sternum) (vertebrae)	* ✓ * ✓	[illegible]
Pituitary		OK.
Lung	*	pale
Heart		✓
Thymus	*	
Liver (3)	*	[illegible]
Gall Bladder		
Spleen	* ✓	[illegible]
Kidney	* ✓	pale
Adrenal Glands (2)	* ✓	pale.
Gastrointestinal		
Bladder		
Anus		
Testis/Ovary		

SAMPLE #: 601	SET	TREATMENT	CAGE	MOUSE	ML	CAUSE-OF-DEATH
	09	XRAY3	11	1	F	14Hdce

B M L L S K A 1 2 3
KnQ PnAhFnPaP

DOB: 86.10.18 DOT: 87.01.23 DOD: 88.03.18

ANEMIC, DYS. DP. SHOCK. HEMOPERITONIUM. WBC:6800 RBC: 1.98M MARROW ?-COMPARTMENTS RED, RIBS WHITE. LUNGS APPEAR PALE. LIVER-1 LOBE RAISED GREEN, FIRM MASS. 1 LOBE DARK RED, ATTACHED BY STALK, 3*5CM RED, FIRM IRREGULAR MASS, REMAINING LOBES ENLARGED, PALE. SPLEEN SL. ENLARGED, RED SMOOTH. KIDNEYS PALE. AD GL PALE. liver- extensive hemorrhage + coagulation necrosis, diffuse infiltration with fusiform cells. kidney- mineralized casts in few tubules. spleen, lung- nvl dx: hemangiosarcoma

SAMPLE #: 601	SET	TREATMENT	CAGE	MOUSE	ML	CAUSE-OF-DEATH
	09	XRAYS	11	1	F	13Jdce

DOB: 86.10.18 DOT: 87.01.23 DOD: 88.03.18
AGE (DAYS): 516

ANEMIC, DYS. DF. SHOCK. HEMOPERITONIUM. WBC:6800 RBC: 1.98M MARROW 2-COMPARTMENTS RED, RIBS WHITE. LUNGS APPEAR PALE. LIVER-1 LOBE RAISED GREEN, FIRM MASS. 1 LOBE DARK RED, ATTACHED BY STALK, 3*5CM RED, FIRM IRREGULAR MASS. REMAINING LOBES ENLARGED, PALE. SPLEEN SL. ENLARGED, RED SMOOTH. KIDNEYS PALE. AD GL PALE. DX:PLT

Liver Extensive hemorrhage + coagulation necrosis - Diffuse infiltration with fusiform cells

Kidney - Mineralized casts in a few tubules

Spleen, Lung, NVL

Dx Hemangiosarcoma (liver)

ANIMAL IDENTIFICATION

Sample No.: 3458	HISTORY and MISCELLANEOUS INFORMATION
Treatment: X 3	
DOB: 8610/13/77	
Set: 9	
Cage: 8 (23)	[illegible]
Animal: 2	[illegible]
Disposition: D=dead, E=euth, A=acc. m=morib, S=suffers	
Date: 82/10/14	

GROSS FINDINGS — Necropsy by: ______ Histology ___ taken ___ missing

Carcass Condition	*	
Blood	*	WBC: 7,300 Hgb: 14.4
Lym. Nd (Cerv.) (Inguin.) (Axill.)(Mesent.)		
Marrow (Sternum) (vertebrae)	* *	
Pituitary		
Lung	*	[illegible]
Heart		~
Thymus	*	~
Liver	*	[illegible]
Gall Bladder		~
Spleen	*	Mild [illegible]
Kidney	*	L- [illegible] pale; R - n.f.
Adrenal Glands (2)	*	~
Gastrointestinal	*	[illegible]
Bladder		~
Anus		~
Testis/Ovary		~
Sem.Vas./Uterus		~
Ext. Genitalia		~

SAMPLE #:3458	SET	TREATMENT	CAGE	MOUSE	ML	CAUSE-OF-DEATH
B M L L S K A 1 2 3	09	XRAY3	6	2	F	14TKzdc
NnT TcFdBnNaNnGn	DOB: 86.10.15		DOT: 87.01.23		DOD: 88.10.14	

POTBELLY, CONGESTED FEET, SL DYS, HUNCHED. WBC:7.300 HGB;14.4 LUNGS 1 0.5CM DIAM RAISED FIRM WELL CIRCUMSCRIBED TAN MASS IN CAUDAL RT LOBE, REST OF THIS LOBE CONSOLIDATED, SL ENLARGED, NOD CONGESTED THROUGHOUT REST OF LUNG. LIVER S
%
LIVER INVOLVED IN T.V.T, SAMPLED 2 AREAS + REMAINING SMALL NORMAL TISSUE. SPLE
N
SL ENLARGED, PGC. KIDNEYS LT-CENTRAL 1/3 PALE RT-NAF AD GL NAF GI-PROM PP(1)

blood smear, spleen, intestine, adrenal-nvl kidney- mineralized casts

liver/lung/dx: hepatoma / pulmonary carcinoma

SAMPLE #:3458	SET	TREATMENT	CAGE	MOUSE	ML	CAUSE-OF-DEATH
	09	XRAY3	8	2	F	13NJ=dc

DOB: 86.10.15 DOT: 87.01.23 DOD: 88.10.14
AGE (DAYS): 729

POTBELLY, CONGESTED FEET, SL DYS, HUNCHED. WBC:7.300 HGB;14.4 LUNGS 1 0.5CM DIAM RAISED FIRM WELL CIRCUMSCRIBED TAN MASS IN CAUDAL RT LOBE. REST OF THIS LOBE CONSOLIDATED, SL ENLARGED, NOD CONGESTED THROUGHOUT REST OF LUNG. LIVER 90% LIVER INVOLVED IN T.V.T, SAMPLED 2 AREAS + REMAINING SMALL NORMAL TISSUE. SPLEEN SL ENLARGED, PGC. KIDNEYS LT-CENTRAL 1/3 PALE RT-NAF AD GL NAF GI-PROM PP(1) DX:LUNG T 2-PLT

Kidney - min. casts

Lung - carcinoma

Liver - hepatoma

Spleen, Intestine, Adrenal - NVL

D+ Pulmonary Carcinoma
Hepatoma

ANIMAL IDENTIFICATION

Sample No.: 3947	HISTORY and MISCELLANEOUS INFORMATION
Treatment: H70-2	lumbed !
DOB: 86·7·22	wnghcrat !
Set: 2	dp !
Cage: 13 (14)	
Animal: 7	
Disposition: D=dead, E=euth, A=acc. m-morib, S=suffers	
Date: 88·11·25	

GROSS FINDINGS Necropsy by: JM Histology ___ taken ___ missing

Carcass Condition	*	clear !
Blood	✓	WBC: 3,500 Hgb: 13.2
Lym. Nd (Cerv.) (Inguin.) (Axill.)(Mesent.)		
Marrow (Sternum) (vertebrae)	✓ ✓	clash
Pituitary		oK !
Lung	✓	mottled !
Heart	✓	
Thymus	*	
Liver	✓	1 x 0.5 cm soft vascular lower right lobe !
Gall Bladder		
Spleen	✓	N.A.F.
Kidney (2)	✓	tan opaq ! with some red streaks !
Adrenal Glands (2)	✓	
Gastrointestinal		
Bladder		
Anus		
Testis/Ovary		

SAMPLE #:3947	SET	TREATMENT	CAGE	MOUSE	ML	CAUSE-OF-DEATH
	02	HTO2	13	7	F	14zTWKc

B M L L S K A 1 2 3
LnN McTdNnBaNnEd

DOB: 86.07.22 DOT: 86.10.27 DOD: 86.11.25

HUNCHED, ROUGH COAT, DP DH. WBC:3.500 HGB:13.2 MARROW DARK. LUNGS MOTTELD. LIVR
1.0*0.5CM T.V.T SPLEEN NAF KIDNEY TAN AREAS. AD GL NAF. blood smear, adrenal, spleen-nvl kidney- mineralized casts lung/liver/harderian/dx: pulmonary c -inoma / hepatoma / harderian gland adenoma

SAMPLE #:3947	SET	TREATMENT	CAGE	MOUSE	ML	CAUSE-OF-DEATH
	02	HT02	13	7	F	13:Jc

DOB: 86.07.22 DOT: 86.10.27 DOD: 88.11.25
AGE (DAYS): 856

HUNCHED, ROUGH COAT, DP DH. WBC:3.500 HGB:13.2 MARROW DARK. LUNGS MOTTELD. LIVER 1.0*0.5CM T.V.T SPLEEN NAF KIDNEY TAN AREAS. AD GL NAF. DX:PLT

Lung - Carcinoma
Liver - hepatoma
Harderian - adenoma
Adrenal, spleen, - NVL
Lung, kidney - min. casts

Dx Pulmonary Carcinoma
Hepatoma
Harderian Gland Adenoma

ANIMAL IDENTIFICATION

	HISTORY and MISCELLANEOUS INFORMATION
Sample No.: 4473	
Treatment: Y-3	
DOB: 86-10-15-16	
Set: 9	
Cage: 7 (23)	
Animal: 6	
Disposition: D=dead, E=euth, A=acc. M=morib, S=suffers	Dm. c
Date: 89.1.22	

GROSS FINDINGS — Necropsy by: J — Histology ___ taken ___ missing

Carcass Condition	•	4.6 hr.
Blood	•	WBC: Hgb:
Lym. Nd (Cerv.) (Inguin.) (Axill.)(Mesent.)		
Marrow (Sternum) (vertebrae)	•	[illegible] pale/red
Pituitary		ok
Lung	•	[illegible] congested [illegible]
Heart		
Thymus	•	
Liver (1)	•	[illegible]
Gall Bladder		
Spleen		[illegible] congested [illegible]
Kidney		congested
Adrenal Glands (2)		NAF (bil)
Gastrointestinal		[illegible] NAF [illegible]
Bladder		
Anus		

SAMPLE #:4473	SET	TREATMENT	CAGE	MOUSE	ML	CAUSE-OF-DEATH
	09	XRAY3	9	6	F	74N

E M L L S K A 1 2 3
MnFpAnFnAaNn

DOB: 86.10.16 DOT: 87.01.23 DOD: 89.01.22

DIMOC 4-6HRS. MARROW SELECTIVE NECROSIS PALE/RED. LUNGS RT LUNGS FIRM, SEV CONGESTED, LT MOTTLED. LIVER SEV CONGESTED SPLEEN SL ENLARGED, CONGESTED, RANDOM DARK AREAS. KIDNEY CONGESTED. AD GL NAF GI-MOD AUTOLYSIS, MASSIVE PINWORMS. bone marrow, spleen, adrenal, liver-nvl kidney- mineralized casts lung/dx: suppurative b-pneumonia

APPENDIX C

CODES USED TO CONVERT HANDWRITTEN DIAGNOSES TO A COMPUTER RECORD, PLUS EXAMPLES OF THE PRINTOUTS OF THE COMPUTER RECORDS

(Note: In the computer printout, D-O-B indicates date of birth, D-O-D is date of death and C-O-D stands for cause of death. S# is a sequential number assigned to each mouse at death. The fields labelled organ flags are as follows in sequence: Blood, Marrow, Lung, Liver, Spleen, Kidney and Adrenal Gland, while 1, 2 and 3 were spaces for other tissues such as lymph node, eye or harderian gland. The top line on the printout indicates the group, date of treatment and radiation dose in Gy for all animals on the page.)

CET: 02 TREATMENT: HT02 DATE OF TREATMENT: 86.10.27 DOSE: 1.01 SUBPAGE: 0

CAGE	D-O-B	#	D-O-D	ML	COD	S#	ORGAN FLAGS B	M	L	L	S	K	A	1	2	3	AGE(D)
13	86.07.22	1	87.11.27	F	47K	190		Nn	Nn	Tc	Fn	Nn	N				492
13	86.07.22	2	89.02.17	F	14Kbe	437	Kn	Pn	Nn	Tc	Mn	Pg	Wn				574
13	86.07.22	3	88.06.02	F	14Kbd	1286	Jn	N	Cn	Tc	Fn	Ga	Gn				680
13	86.07.22	4	88.07.27	F	14YZbdc	2185		N	Nn	Nn	Gn	Ba	Nn	GK			705
13	86.07.22	5	88.09.08	F	14KTc	2948	Nn	N	Tc	Td	Fn	Pa	Nn				778
13	86.07.22	6	88.10.08	F	14KUzc	3385	Nn	N	Nn	Td	Fn	Pn	Nn	L	GA	E	808
13	86.07.22	7	88.11.25	F	14zTWkc	3947	Ln	N	Mc	Td	Nn	Ba	Nn	Ed			856
14	86.07.23	1	88.06.14	F	14zOc	1467	Nr	N	Hn	En	Gk	Hh	Gn				691
14	86.07.23	2	88.09.14	F	14TKbc	3069	Nn	N	Tc	Td	Gn	Ba	Nn	Sn			783
14	86.07.23	3	88.09.22	T	14STc	3175	Na	Mm	Tc	Em	Dm	Pa	Nm				791
14	86.07.23	4	88.12.12	F	14TJzdc	4127	Nn	N	Rc	TA	Rn	Pa	Nn	H			872
14	86.07.23	5	89.01.03	F	140Jz	4330	Ln	N	Cn	TA	Gn	Pa	Nn	Gn			894
14	86.07.23	6	89.02.06	F	14zJc	4571	Ln	N	Cn	TA	Fn	Pa	Nn				928
14	86.07.23	7	89.03.09	F	14zYZc	4729	Ln	N	Cn	Nn	Nn	Ba	Nn	Un			959
15	86.07.24	1	88.07.08	F	14Jzc	1853	Nn	N	Hn	TA	Mn	Pa	Nn	L			714
15	86.07.24	2	88.08.10	F	14Jcd	2401	Ln	N	Nn	TA	Gn	Aa	Nn	Gn			747
15	86.07.24	3	88.08.22	F	140zc	2600	Ln	N	Nn	Nn	Gn	Pi	Gn				759
15	86.07.24	4	88.09.08	F	14Jc	2950	Ln	N	Mn	PA	Gn	Pa	Nn	Un			776
15	86.07.24	5	88.10.02	F	14zKNdc	3290	Nn	N	AA	Td	Gn	Pa	Wn	Hn			800
15	86.07.24	6	88.11.11	F	14K	3787	Nn	N	Cn	Td	Pn	Ba	Nn				840
15	86.07.24	7	88.12.01	F	14Tc	4004	Ln	N	Tc	Nn	Gn	Bi	Nn	G			860
16	86.07.25	1	87.09.25	F	470	85		Nj	Nn	Nn	Nn	Hn	Nn				426
16	86.07.25	2	88.01.23	F	47Kb	327		Pn	Nn	Td	Nn	Na	Nn				546
16	86.07.25	3	88.06.17	T	14Szbc	1502	G	Wm	Rm	Im	Dm	Pm	Pm				692
16	86.07.25	4	88.06.27	F	14zKc	1678	Nn	N	En	Td	Nn	Nf	Nn				702
16	86.07.25	5	88.06.29	F	14Kcd	1735	Nn	N	Pn	Td	Fn	Pa	Nn				704
16	86.07.25	6	88.07.06	F	14zKc	1825	Jn	N	Hn	Td	Fn	Ba	Nn				711
16	86.07.25	7	88.11.03	F	14W	3695	Nn	N	Mn	Tn	Nn	Aa	Na	Ed			831
17	86.07.26	1	87.10.07	F	124Lc	91	Fl	Pl	Nl	El	El	Nl	Nl				437
17	86.07.26	2	87.11.03	F	47Wj	136		Nn	Nn	Jn	Mn	Fn	N				464
17	86.07.26	3	88.03.14	F	14Re	578	Kn	Pn	Pn	En	Er	Pa	P	Lr			596
17	86.07.26	4	88.06.07	F	14zKc	1354	Nn	N	Hn	Ed	Nn	Pa	Gn	Gn			681
17	86.07.26	5	88.08.09	F	14zHdce	2391	Kn	N	Hn	Th	Mh	Pa	Wn	H			744
17	86.07.26	6	88.08.17	F	14Jc	2512		N	Mn	TA	Gn	Ai	Nn				752
17	86.07.26	7	88.09.12	F	14Hdbc	3020	Kb	N	Nn	Ah	Fn	Pa	Pn	M			778
17	86.07.26	8	88.09.26	F	14KMbc	3216	Nn	N	MA	Tc	Fn	Pa	Nn				792
18	86.07.29	1	87.11.14	F	47KN	157		Nn	Np	Td	Fn	Ei	N				472
18	86.07.29	2	88.07.13	F	14YZdc	1934	Ln	N	Mn	Pn	Gn	Na	Nn				714
18	86.07.29	3	88.08.21	F	14KTc	2575	Nn	N	Rc	Tc	Gn	Pa	Gn				753
18	86.07.29	4	88.08.29	F	14KTc	2745	Ln	N	Mc	Tc	Pn	Na	Nn				761
18	86.07.29	5	88.09.06	F	14KWTzbI	2900	Nn	N	Rd	Td	Gn	Pa	Nn	Ed			769
18	86.07.29	6	89.01.27	F	14TKzc	4518	Mn	N	Tc	Td	Gn	Na	Nn				912
18	86.07.29	7	89.01.27	F	140z	4519	Ln	N	Tn	Nn	Gn	Ah	Nn				912

REPORT #2: FULL FILE LISTING

SET: 09 TREATMENT: XRAY3 DATE OF TREATMENT: 87.01.23 DOSE: 2.87 SUBPAGE: 2

CAGE	D-O-B	#	D-O-D	ML	COD	S#	ORGAN FLAGS B	L	L	S	K	A	1	2	3	AGE(D)
7	86.10.14	1	88.05.31	F	74Kzx	1242	N	x	xn	Td	xn	xn				594
7	86.10.14	2	88.07.15	F	14TKc	1977	Jr	N	Rc	Td	Fn	Ba	Nn			639
7	86.10.14	3	88.09.17	F	14YZzbac	3119	Nn	N	Rn	Af	Mk	Pa	Nn	Lk		703
7	86.10.14	4	88.10.11	F	14HKzb	3406	Jn	N	Rn	Td	Mn	Na	Nn			727
7	86.10.14	5	88.12.07	F	14TrC	4070	Nn	N	Md	n	Mr	Pr	Nn	Lr		784
7	86.10.14	6	88.12.23	F	14TJz	4249	Nn	Pj	Ac	TA	Mn	Pn	Nn			800
7	86.10.14	7	89.01.28	F	14Jz	4525	Nn	N	Nn	TA	Gn	Aa	Nn	En		836
8	86.10.15	1	88.08.22	F	14Kzc	2589	Nn	N	Nn	Td	Mn	Nn	Nn			676
8	86.10.15	2	88.10.14	F	14TKzdc	3458	Nn	T	Tc	Fd	Bn	Na	Nn	Gn		729
8	86.10.15	3	88.11.11	F	14Hz	3785	Nn	N	Nn	Pn	An	Pa	Nn	Mh		757
8	86.10.15	4	88.11.15	F	14K	3843	Nn	N	Mn	Td	Fn	Na	Nn			761
8	86.10.15	5	88.11.30	F	14zT	3998	Nn	N	Rc	Nn	Nn	Nn	Nn	U		776
8	86.10.15	6	89.01.12	F	14J	4403	Ln	N	Cn	TA	Gn	Pa	Nn			819
8	86.10.15	7	89.02.27	F	74YZ	4670		Nn	Dn	Tn	Nn	Na	An			865
9	86.10.16	1	88.07.01	F	14Nzc	1763	Nn	N	Jp	Nn	Fn	Aa	Nn	Gk		623
9	86.10.16	2	88.07.15	F	14KW	1975	Jn	Nn	Mn	Tc	Fn	Ga	Nn	Ed		637
9	86.10.16	3	88.12.12	F	14KNzc	4120	Mn	N	Kp	Tc	Gn	Pa	Nn	Ga		787
9	86.10.16	4	88.12.23	F	14KMz	4250	Ln	N	CA	Tc	Fn	An	Nn	S		798
9	86.10.16	5	89.01.20	F	14TKzc	4462		N	Td	Tc	Gn	Pa	Nn	Gn	An	Hn 826
9	86.10.16	6	89.01.22	F	74N	4473		Mn	Fp	An	Fn	Aa	Nn			828
9	86.10.16	7	89.01.28	F	14TNzd	4524	Jn	F	Fc	Nn	Fn	Na	Nn	Gn	T	834
10	86.10.17	1	88.04.24	F	47zYZv	901		Nn	Nn	En	Pn	Ba	Nn			554
10	86.10.17	2	88.08.12	F	14zKc	2445	Ln	N	Nn	Td	Nn	Na	Nn			664
10	86.10.17	3	88.08.15	F	13Jc	2477		N	M	T	G	G	G			667
10	86.10.17	4	88.09.15	F	14NKbd	3082	Jn	N	Fp	Rd	Nn	Na	Nn			698
10	86.10.17	5	88.10.12	F	14WKNbc	3425	Ln	An	Kp	Rd	Fn	Nn	Nn	Ed		725
10	86.10.17	6	88.10.13	F	14TNc	3440	Mn	N	Tc	Tn	Fn	Na	Nn			726
10	86.10.17	7	88.11.11	F	14TJN	3797	Ln	N	Tc	RA	Gn	An	Nn	L		755
11	86.10.18	1	88.03.18	F	14Hdce	601	Kn	Q	Pn	Ah	Fn	Pa	P			516
11	86.10.18	2	88.06.02	F	14Ksbd	1281	Nn	N	Nn	Td	Mn	Ba	Nn	L	Gn	592
11	86.10.18	3	88.06.15	F	14zK	1485	Ln	N	Hn	Td	Gn	Nn	Nn			605
11	86.10.18	4	88.09.16	F	14Jzc	3109	Nn	N	Nn	TA	Fk	Na	Nn	Gn	L	698
11	86.10.18	5	88.10.17	F	140Uzc	3479	Fn	Pj	Cn	Es	F	AD	N	Ui	G	E 729
11	86.10.18	6	88.11.16	F	14WJc	3852	Ln	N	Cn	Aa	Fn	Na		Ed		759
11	86.10.18	7	89.01.24	F	14Kz	4493	Nn	N	Tn	Td	Fn	Pa	Nn	Gn		828
12	86.10.18	1	87.11.25	F	47Hs	184		Nn	Dn	Tq	Fn	Nn	Nn			402
12	86.10.18	2	88.08.11	F	14Jbc	2415	Ln	N	Mn	RA	Nn	Aa	Nn	Un		662
12	86.10.18	3	88.08.17	F	14Jcb	2501	Kn	N	Mn	TA	Pn	Pa	Pn			66E
12	86.10.18	4	88.10.14	F	14Kzc	3449	Jn	N	Cn	Td	Fn	Na	Nn			72E
12	86.10.18	5	88.12.21	F	14H	4227	Ln	N	Cn	Ah	Gn	Na	Nn	Gn		79<
12	86.10.18	6	89.03.03	F	14Tzbc	4693	Jn	N	Tc	TA	Nn	Pa	Nn	Gn		86E
12	86.10.18	7	89.03.03	F	14Hzb	4694	Jn	N	Nn	Ah	Nn	Pa	Nn	Ln	Gn	86E

FIELD: COD

1 - EUTHANIZED

2 - MORIBUND/SUFFERING

3 - GROSS TENTATIVE DIAGNOSIS

4 - DIAGNOSIS BOTH GROSS + HISTO

5 - UNKNOWN DIAGNOSIS

6 -

7 - FOUND DEAD

8 - FATAL TRAUMATIC ACCIDENT

9 - ESCAPED

A - SEE MEMO	a - ANOREXIC
B - MYELOID HYPERPLASIA	b - DEHYDRATE
C - LYMPHOID HYPERPLASIA	c - DEPRESSED
D - ADENOMA	d - DYSNEA
E - ADENOCARCINOMA	e - ANEMIA
F - FIBROSARCOMA	f -
G - TORTICOLLIS	g -
H - HEMANGIOSARCOMA	h - HEMORRHAGE
J - LIVER DISORDER	j - CARCASS JAUNDICED
K - LIVER TUMOR	k -
L - LYMPHOMA	l - HAIR LOSS (ALOPECIA)
M - METASTASIS	m -
N - LUNG DISORDER	n -
T - LUNG TUMOR	
O - KIDNEY DISORDER	o -
P - KIDNEY TUMOR	P - PARALYSIS

R - RETICULUM CELL SARCOMA

S - SUSPICIOUS/CONFIRMED FOR M.L.

U - OTHER DISORDER

W - OTHER TUMOR

X - NO SAMPLES

Y - NOGA NO EVIDENCE OF MALIGNANCY

Z - CONSIDER M.L. NEGATIVE

r -

s - ASCITES

t - THORACIC FLUID

u - DISTENDID BLADDER

w - SEVERE AUTOLYSIS

x - MODERATE AUTOLYSIS

y - EXPANDED FIELD IN MEM

z - AUTOPSY PERFORMED BY @

@ -AMF/JM z BEFORE DX
-HW z AFTER DX
-NJG/JSJ NO z

* QUERY INTERPRETATION OF EAR TAG

$ - DEATH DUE TO OTHER CAUSES, SUGGESTIVE OF M.L.

+ - SEE COMMENT IN MEMO

FIELD : BLOOD

A - SEE MEMO	a - POOR SMEAR, INCONCLUSIVE
B -	b -
C -	c -
D -	d -
E - ↓ WBC, ↓ RBC	e - EARLY ONSET M.L.
F - ↑ WBC, NORMAL RBC	f -
G - ↑ WBC, ↓ RBC	g -
H - ↑ WBC, ↑ RBC	h -
I -	i -
J - NORMAL WBC, ↑ RBC	j - MYELOID HYPERPLASIA
K - NORMAL WBC, ↓ RBC	k - LYMPHOID HYPERPLASIA
L - √ WBC, NORMAL RBC	l - NEOPLASTIC LYMPHOCYTES
M - √ WBC, ↑ RBC	m - NEOPLASTIC MYELOCYTES
N - NORMAL WBC, NORMAL RBC	n - APPEARS NORMAL
O -	o -
P - EXTREME POLYCYTEMIA	p -
Q - QUESTIONABLE	q -
R -	r - [illegible]
S -	s - LEFT SHIFT NEUTROPHILS
T -	t -
U -	u -
V -	v -
W -	x -
Y -	y -

FIELD : MARROW

A - SEE MEMO	a -
B -	b -
C -	c -
D -	d -
E -	e -
F -	f -
G -	g -
H -	h -
I -	i - INFLAMMATORY
J -	j - MYELOID HYPERPLASIA
K -	k - LYMPHOID HYPERPLASIA
L -	l - NEOPLASTIC LYMPHOCYTES
M - MIXTURE RED + PALE MARROW	m - NEOPLASTIC MYELOID CELLS
N - NORMAL	n - APPEARS NORMAL
O - MIXTURE PALE + WHITE MARROW	o - OTHER
P - PALE	p -
Q - QUESTIONABLE	q - MESENCHYMAL CELLS
R -	r - RETICULUM CELLS
S - SLIGHTLY PALE	s - NECROSIS
T - PALE TO WHITE IN COLOR	t -
U -	u -
V -	v -
W - WHITE	w -
X -	x - MOD AUTOLYSIS
	y - SEV AUTOLYSIS

50/51

FIELD : LUNG

A - SEE MEMO	a -PULMONARY ABCESS
B - ABSCESS	b -
C - CONGESTED	c - PULMONARY CARCINOMA/ADENOCARCINOMA
D - COLLAPSED/CONSOLIDATED	d - ADENOMA (CUBOIDAL EPITHELIUM)
E - ENLARGED	e -
F - FIRM/ENLARGED	f - FOCAL ALVEOLITIS
G - RED FOCI	g -
H - HEMORRHAGE	h -
I - INFILTRATED	i - INFLAMATORY
J - ATELECTATIC	j - MYELOID HYPERPLASIA
K - CONSOLIDATED	k - LYMPHOID HYPERPLASIA
L -	l - NEOPLASTIC LYMPHOCYTES
M - MOTTLED	m - NEOPLASTIC MYELOID CELLS
N - NORMAL	n - APPEARS NORMAL
O - MOD ENLARGED	o - OTHER
P - PALE	p - PNEUMONIA
Q - QUESTIONABLE	q - MESENCHYMAL CELLS
R - NODULAR TUMOR(S)	r - RETICULUM CELLS
S - SMALL	s - NECROSIS
T - WELL CIRCUMSCRIBED TUMOR IN LUNG	t -
U - DIFFUSE + NODULAR TUMORS	u -
V - FIRM WITH DIFFUSE INFILTRATION	v -
X -	x - MOD AUTOLYSIS
Y -	y - SEV AUTOLYSIS
Z -	z -

93/5

FIELD: SPLEEN

A - SEE MEMO	a - abscessation
B - WHITE PATCHES	b -
C - CONGESTED	c -
D - MARKEDLY ENLARGED, PALE, FRAGILE	d - ↑ ERYTHROPOIESIS
E - MARKEDLY ENLARGED, RED, FRAGILE	e -
F - SLIGHTLY ENLARGED	f -
G - PROMINENT GERMINAL CENTERS	g -
H -	h - HEMANGIOSARCOMA
I - DIFFUSELY INFILTRATED	i - INFLAMMATION
J -	j - MYELOID HYPERPLASIA
K -	k - LYMPHOID HYPERPLASIA
L -	l - NEOPLASTIC LYMPHOCYTES
M - MODERATELY ENLARGED	m - NEOPLASITC MYELOID CELLS
N - NORMAL	n - NORMAL
O -	o - OTHER
P - PALE, NORMAL - SLIGHTLY ENLARGED	p - PMN'S
Q - QUESTIONABLE	q - MESENCHYMAL CELLLS
R - NODULAR TUMOR(S)	r - RETICULUM CELLS
S - SMALL	s - NECROSIS
T -	t -
U - ENLARGED WITH NODULAR TUMORS	u -
V -	v -
X -	x - MOD AUTOLYSIS
Y -	y - SEV AUTOLYSIS
Z -	z -

9x01

FIELD : LIVER

A - SEE MEMO	a - BILIARY CYSTS
B - ADHESIONS	b - BILIARY DUCT CARCINOMA
C - FIRM	c - CARCINOMA (POORLY DIFF. HEPATOCYTES
D - FRAGILE	d - HEPATOMA (WELL DIFF. HEPATOCYTES)
E - ENLARGED	e -
F - SLIGHTLY ENLARGED	f - fibrosis
G - RED PETECHAE	g - HEPATITIS
H - ENLARGED: BILE BLOCKAGE, BILE ON CUT SURFACE	h - HEMANGIOSARCOMA
I - INFILTRATED, DIFFUSELY	i - INFARCTION
J - JAUNDICE	j - MYELOID HYPERPLASIA
K - PROMINENT LOBULES	k - LYMPHOID HYPERPLASIA
L - PROMINENT LOBULES; JAUNDICE	l - NEOPLASTIC LYMPHOCYTES
M - PALE, PROMINENT LOBULES	m - NEOPLASTIC MYELOID CELLS
N - NORMAL	n - NORMAL
O - MODERATELY ENLARGED	o - OTHER
P - PALE	p - PMN'S
Q - QUESTIONABLE	q - MESENCHYMAL CELLS.
R - NODULE TUMOR(S)	r - RETICULUM CELLS
S - SMALL	s - NECROSIS
T - ~~WELL CIRCUMSCRIBED~~ TUMOR IN ~~LIVER~~	t -
U - DIFFUSE AND NODULAR TUMOR(S)	u -
V -	v -
x -	x - MOD AUTOLYSIS
Y -	y - SEV AUTOLYSIS

FIELD : KIDNEYS

A - SEE MEMO	a - CASTS 1-PROTEIN 2-MINERALIZED
B - TAN/PALE AREAS	b - BLOCKAGE
C - CYSTITIS	c - RENAL CARCINOMA
D - NUMEROUS WHITE FOCI	d - NEPHROSIS, FOCAL
E - ENLARGED, BOTH	e -
F - ENLARGED, RIGHT/LEFT	f -
G - RED PETECHAE/FOCI	g - GLOMERULOPATHY
H - HYDRONEPHROSIS	h - HYDRONEPHROSIS
I - ENLARGED WITH WHITE FOCI	i - INFLAMMATORY
J - JAUNDICED	j - MYELOID HYPERPLASIA
K - ENLARGED WITH RED FOCI	k - LYMPHOID HYPERPLASIA
L -	l - NEOPLASTIC LYMPHOCYTES
M -	m - NEOPLASTIC MYELOID CELLS
N - NORMAL	n - APPEARS NORMAL
O - PYELONEPHRITIS	o - OTHER
P - PALE	p - PMN'S
Q - QUESTIONABLE	q - MESENCHYMAL CELLS
R - NODULAR TUMOR(S)	r - RETICULUM CELLS
S - SMALL, BOTH	s - NECROSIS
T - SMALL, RIGHT/LEFT	t - THICKENED BASEMENT MEMBRANES
U - URETHRAL BLOCKAGE	u -
V - ENLARGED WITH NODULAR TUMORS	v -
X - ENLARGED, PALE, WHITE FOCAL TUMORS (WHITE PATCHES)	x - MOD AUTOLYSIS
Y -	y - SEV AUTOLYSIS

FIELD : ADRENAL GLAND

A - SEE MEMO	a -
B -	b - C-carcinoma
C - ENLARGED, PALE, WHITE FOCI	c - ADENOMA (CORTICAL)
D - ENLARGED, PALE	d - CYST (CORTICAL)
E - ENLARGED, BOTH	e -
F - ENLARGED, RIGHT/LEFT	f -
G - ENLARGED, WHITE FOCI	g -
H - CLEAR FOCI	h - Hyperplasia, nodular
I -	i - INFLAMMATORY
J -	j - MYELOID HYPERPLASIA
K -	k - LYMPHOID HYPERPLASIA
L -	l - NEOPLASTIC LYMPHOCYTES
M -	m - NEOPLASTIC MYELOID CELLS
N - NORMAL	n - APPEARS NORMAL
O - PALE TO WHITE IN COLOR	o - OTHER
P - PALE	p - PMN'S
Q - QUESTIONABLE	q - MESENCHYMAL CELLS
R -	r - RETICULUM CELLS
S - SMALL, BOTH	s - NECROSIS
T - SMALL, RIGHT/LEFT	t - subcapsular adenoma (cortical)
U -	u -
V -	v -
W - WHITE	x - MOD AUTOLYSIS
X -	Y - SEV AUTOLYSIS

FIELD : OTHER

A - SEE MEMO	a -
B - RIBS	b -
C -	c - SQUAMOUS CELL CARCINOMA
D - STOMACH	d -
E - EYELIDS/MASS	e -
F - EYE ORBITAL/MASS	f -
G - GI TRACT	g -
H - HEART	h -
I - URETER ,BLADDER	i - INFLAMMATORY
J -	j - MYELOID HYPERPLASIA
K -	k - LYMPHOID HYPERPLASIA
L - LYMPH NODE	l - NEOPLASTIC LYMPHOCYTES
M - MASS	m - NEOPLASTIC MYELOID CELLS
N -	n - ADENOCARCINOMA
O - PITUITARY TUMOR	o -
P - PANCREAS	p -
Q -	q - MESENCHYMAL CELLS
R - TESTIS	r - RETICULUM CELLS
S - SEMINAL VESICLE	s - DEGENERATION
T - THYMUS	t -
U - URINARY BLADDER DISTENDED	u -
V - PROSTATE	v -
X -	x - MOD AUTOLYSIS
Y -	y - SEV AUTOLYSIS
Z -	z -

APPENDIX D

CALCULATION OF RBE VALUES AND THEIR STANDARD DEVIATIONS

For linear, non-threshold plots of effects against dose, the relative biological effectiveness (RBE) of tritium beta-rays compared to X-rays is given simply by the ratio b_2/b_1, where b_2 and b_1 are the slopes of the straight lines for effect per unit dose for HTO and for X-rays, respectively. Assuming a normal distribution of RBE values, the standard deviation of the RBE is given by the formula [4]

$$\frac{b_2}{b_1}\sqrt{\left(\frac{\Delta b_2}{b_2}\right)^2 + \left(\frac{\Delta b_1}{b_1}\right)^2}$$

where Δb_2 is the standard deviation of slope b_2
and Δb_1 is the standard deviation of b_1.

APPENDIX E

AECL STANDARD POLICIES
AND PROCEDURES
CHALK RIVER SERIES. A-10

Atomic Energy of Canada Limited

Research Company

STANDARD POLICIES AND PROCEDURES

CHALK RIVER SERIES

A-10

1984 March

CRNL - ANIMAL CARE COMMITTEE

1. GENERAL

The CRNL - Animal Care Committee (CRNL-ACC) shall operate in compliance with the "Animals for Research Act" (RSO 1970, Chapter 22, amended 1972 December) which is the primary legislation governing the use of animals for research and the operation of research animal facilities in Ontario. This Act is administered by the Veterinary Services Branch of the Provincial Ministry of Agriculture.

The Canadian Council on Animal Care (CCAC) (an autonomous advisory and supervisory body operating under the aegis of the Association of Universities and Colleges of Canada) makes a site visit to CRNL approximately each three years to assess the Animal Facility and procedures employed in animal based research. The CRNL-ACC has adopted officially the "CCAC Guide to the Care and Use of Experimental Animals" as CRNL policy for all animal research. An "animal" to which the following terms of reference apply will be defined as a vertebrate.

2. TERMS OF REFERENCE OF THE CRNL-ANIMAL CARE COMMITTEE

a) Authority

No animal research or testing project, including field studies involving animals, should be commenced without prior approval of the CRNL-ACC.

The CRNL-ACC shall have the authority:

i) to stop any objectionable procedure, if it considers that unnecessary pain is being experienced by the animal;

ii) to terminate immediately any use of animals which by deviating from the approved proposal causes undue pain and distress to such animals; and

iii) to humanely kill an animal if the committee feels that pain or distress caused to the animal cannot be alleviated.

b) Responsibility

The CRNL-ACC has the responsibility:

i) to review and assess all proposed animal based research projects with particular emphasis on the "Ethics of Animal Experimentation" as published by the Canadian Council on Animal Care and where necessary, require further supportive information from the investigator;

ii) to assure that all animal users have the opportunity to become familiar with CCAC's "Guide" including the "Ethics of Animal Experimentation", provincial or municipal statutes that may apply, and institutional requirements; and

iii) to ensure adequate care of animals in all stages of their life and veterinary assistance in case of sickness, injury and elective procedures.

c) Procedures

In carrying out its duties, the CRNL-ACC shall:

i) require the submission of a written animal use protocol from the investigator for approval by the CRNL-ACC prior to commencement of the project;

ii) make at least one site visit annually as a committee and as often as necessary to fulfill their terms of reference; and

iii) establish procedures to ensure that:

- there is a reasonable expectation that the animal studies in question will contribute significantly to knowledge which may eventually lead to the improvement of the health and welfare of either humans or animals;

- unnecessary pain is avoided;

- anesthesia and analgesia are properly and effectively used where indicated;

- painful studies requiring exemption from the use of either anesthetics or analgesia are subject to

particular scrutiny, not only prior to approval, but during the experiment; and

- post-operative care commensurate with current veterinary concepts is provided.

3. FORM CRNL-3290 (4/82) CRNL - ANIMAL CARE COMMITTEE RESEARCH PROPOSAL (copy attached)

This form is completed by the principal investigator and submitted to the Chairman of the CRNL - ACC at least 2 weeks prior to conducting a new project. In case of a modification to an existing protocol, the Chairman of the CRNL - ACC has the authority to give a 2 week tentative approval of the change to be made in the protocol. The Chairman is responsible to distribute copies of the new (or modified) protocol. Of particular interest to the committee are procedures and manipulations to be performed on live animals. In case of any queries with the proposal, the investigator is contacted through the Chairman concerning the issue. If unresolved, a committee meeting is called to decide on the appropriate course of action.

4. MEMBERSHIP

In as far as possible, the following CRNL representation should be included on the Committee:

1) a qualified veterinarian (Chairman);
2) Head, Radiation Biology Branch;
3) a biological scientist familiar with animal experiments;
4) a biological scientist familiar with environmental experiments;
5) an animal care technologist;
6) a representative from a branch which is not involved in animal experiments.

D.G. Breckon
Manager, Administration Division

NJG/DKM/AMM

New Policy

CRNL 3290 (4-82)

CRNL ANIMAL CARE COMMITTEE RESEARCH PROPOSAL

☐ NEW PROTOCOL ☐ RENEWAL ☐ MODIFICATION	PREVIOUS PROTOCOL NO.	DATE
SENIOR INVESTIGATOR & RANK	PHONE NO. Bus. Res.	BRANCH
PROJECT TITLE		EMERGENCY PHONE NO.(S)
INVESTIGATOR(S) CARRYING OUT RESEARCH & RANK	PHONE NO. Bus. Res.	BRANCH
SUPPORTING AGENCY		

ANIMAL SPECIES:					
APPROX. NUMBER:	PER MONTH	3 YEAR TOTAL	LOCATION OF EXPERIMENT (STATE ROOM NO. & BLDG. NO.)		
TYPE OF EXPERIMENT:	☐ Surgical	☐ Non-Surgical	☐ Acute	☐ Survival	
TYPE OF SURGERY:					
EXPECTED PAIN LEVEL	☐ Nil	☐ Low	☐ Moderate	☐ High	
ANESTHETICS AND/OR ANALGESICS TO BE USED:					
METHODS OF EUTHANASIA TO BE EMPLOYED:	☐ Anesthetic overdose ☐ Cervical Dislocation	☐ Decapitation ☐ Electrocution	☐ Exsanguination with anesthetic ☐ Gas, Ether, CO_2	☐ Pithing ☐ Stunning	☐ Other (Specify)
HAZARDOUS AGENTS TO BE USED	☐ Infectious	☐ Biological	☐ Chemical	☐ Radio Isotopes	NOTE DETAILS OF DOSAGE AND PRECAUTIONS TO BE TAKEN IN OUTLINE BELOW

OUTLINE OF PROCEDURES AND TECHNIQUES TO BE USED:

TRAPPING OR WILDLIFE LICENCES:

DECLARATION: All animals used in this research project will be cared for in accordance with the recommendations of the Canadian Council on animal care, the requirements under "The Animals For Research Act, R.S.O. 1970", and the regulations of the CRNL Animal Care Committee.

PRINCIPAL INVESTIGATOR

ENDORSEMENTS:

COMMITTEE CHAIRMAN — DATE — CRNL VETERINARIAN — DATE

APPENDIX F

EXAMPLES OF THE PERSONNEL SCHEDULE USED FOR EXAMINATION AND POSTMORTEM OF MICE

APRIL 1988

	MON.	TUES.	WED.	THURS.	FRI.
Animal Check	Jim M. /Anne F.	Heather W. /Anne F.	Heather W. /Anne F.	Jim M. /Anne F.	Anne F.
Rooms	1 & 2 /3	3 /1 & 2	2 /1 & 3	1 & 3 /2	1, 2, 3
Necropsy	Anne F.	Heather W.	Heather W.	Anne F.	Anne F.
Blood Analysis	Anne F., Joanne J.	Anne F.	Anne F.	Joanne J.	Anne F.

WEEKENDS MARCH - JUNE 1988

March	5	Joanne J.
	12	Anne F.
	19	Jim M. / Heather W.
	26	Joanne J.
April	2	Anne F.
	9	Jim M. / Heather W.
	16	Joanne J.
	23	Heather W.
	30	Joanne J.
May	7	Anne F.
	14	Joanne J.
	21	Heather W.
	28	Heather W. / Jim M.
June	4	Joanne J.
	11	Anne F.
	18	Heather W.
	25	Joanne J. / Jim M.

WEEKENDS JANUARY - APRIL 1989

Jan.	7	Joanne J.
	14	Heather W.
	21	Jim M.
	28	Heather W.
Feb.	4	Joanne J.
	11	Jim M.
	18	Joanne J.
	25	Heather W.
Mar.	4	Joanne J.
	11	Joanne J.
	18	Jim M.
	25	Heather W.
April	8	Heather W.
	15	Jim M.
	22	Joanne J.
	29	Heather W.

MARCH 1989

	MON	TUES.	WED.	THURS.	FRI.
Animal Check	Heather W. /Joanne J.	Heather W. /Joanne J.	Heather W. /Joanne J.	Anne F. /Joanne J	Anne F. Heather W. Joanne J.
Rooms	1 & 2 /3	3 /1 & 2	2 /1 & 3	1 & 3 /2	1, 2, 3
Necropsy	Joanne J.	Anne F., Heather W.	Anne F., Heather W.	Joanne J.	Joanne J.
Blood Analysis	Anne F., Heather W.	Joanne J.	Joanne J.	Anne F., Heather W	Joanne J.

JULY 1989

	MON.	TUES.	WED.	THURS.	FRI.
Animal Check	Jim M. /Joanne J.	Heather W. /Joanne J.	Heather W. /Joanne J.	Jim M. /Heather W	Joanne J.
Rooms	1 & 2 /3	3 /1 & 2	2 /1 & 3	1 & 3 /2	1, 2, 3
Necropsy	Joanne J.	Heather W.	Heather W.	Jim M., Heather W.	Joanne J.
Blood Analysis	Joanne J.	Joanne J.	Joanne J.	Heather W.	Joanne J.

AUGUST 1989

	MON.	TUES.	WED.	THURS.	FRI.
Animal Check	Heather W. /Joanne J.	Heather W.	Heather W.	Heather W. /Joanne J.	Joanne J
Rooms	1 /2	1 & 2	1 & 2	2 /1	1 & 2
Necropsy	Heather W.	Heather W.	Heather W.	Joanne J.	Joanne J
Blood Analysis	Joanne J.	Joanne J.	Joanne J.	Heather W.	Joanne J

[Note: The total number of animals was sufficiently reduced to allow for a reduction in the number of animal rooms in use from three to two in August 1989.]