CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

PC 117300 007252

JUN 19 1989

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Glyphosate - EPA Registration Nos. 524-318 and 524-333 - Historical Control Data for Mouse Kidney Tumors

MRID No.: 00130406
Caswell No.: 661A
Record No.: 238,412
Project No.: 9-0697

FROM: William Dykstra, Reviewer
Review Section I
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

William Dykstra 6/9/89

TO: Robert J. Taylor, PM 25
Fungicide-Herbicide Branch
Registration Division (H7505C)

THRU: Edwin Budd, Acting Branch Chief
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

and

William Burham, Deputy Director
Health Effects Division (H7509C)

Requested Action

Review historical control data on mouse kidney tumors submitted by Monsanto in response to meeting of November 10, 1988.

Conclusions and Recommendations

The historical control data showed that the incidence of renal neoplasms in male CD-1 mice ranged from 0 to 3.3 percent at Bio/dynamics (the laboratory that performed the glyphosate mouse oncogenicity study), 0 to 4.7 percent at Hazleton, 0 to 1.7 percent at IRDC, 0 to 3.3 percent at Litton Bionetics, and 0 to 1.4 percent in Japan (Japanese Institute for Environmental Toxicology). The range of incidences of 0 to 7.1 percent reported by Monsanto in their November 10, 1988 meeting with the Agency was taken from the data on F_1 male mice in reproduction studies at Hazleton.

These F_1 data could not be further substantiated by Monsanto and therefore, cannot be used to support the Monsanto position.

Other data study presented by Monsanto, briefly, were two chronic bioassays with male CD-1 mice in which the following incidences of renal neoplasms were noted:

	Control	Low	Mid	High
Study I	0/80	2/80	1/80	2/80
Study II	2/50	1/50	3/50	3/50

Monsanto cites these data as showing an incidence of 0 to 6 percent in control or treated groups (the occurrences of renal tumors in treated groups were not considered compound-related) which matches the upper incidence of 6 percent in the glyphosate study. Toxicology Branch (TB) does not consider these random data as convincing.

However, based on a meeting held June 7, 1989 between W. Dykstra, E. Budd, and W. Burnam, TB concludes that a repeat of the mouse oncogenicity study is not required at this time. After the results of the new 2-year rat chronic toxicity and oncogenicity study are reviewed, TB will reconsider whether the repeat of the mouse oncogenicity study is required.

Background

On November 10, 1988, a meeting was held between EPA staff and representatives of Monsanto to discuss the Agency's requirement that the mouse oncogenicity study with glyphosate be repeated (memorandum attached).

Monsanto stated that there were historical control data demonstrating that the incidence of mouse kidney neoplasms ranged from 0 to 7.1 percent. This incidence exceeded the incidence of 6 percent from the high-dose group in the glyphosate study. Monsanto indicated that a repeat mouse oncogenicity study was not required.

EPA stated that the historical control data should be submitted in order to reevaluate the Agency's position on the repeat study.

In response to this request, Monsanto has submitted historical control data from several sources to substantiate their contention regarding the range of mouse kidney tumor neoplasms.

Review

1. The incidence of renal tubule tumors in the glyphosate mouse study is shown below:

	Mouse Kidney			
Dose (ppm)	0	1000	5000	30,000
No. Examined	49	49	50	50
Tubular Adenomas	1	0	1	3
Percent Incidence	2%	0%	2%	6%

2. The historical control data are presented below and are also attached to this memorandum.

   a. Bio/dynamics Historical Control Data - From studies initiated between 1976 and 1980 and terminated between 1978 and 1982, the incidence of tumors is shown below as submitted by Monsanto:

CD-1 COBS (ICR Derived) Mice
Bio/dynamics, Inc.
MALES - KIDNEYS

CONTROL DATA

STUDY I.D.	A	B	C	D	E	F	G	H*	I	J**	K+	L	M**	N	O	P
Tissue/Finding																
No. Examined	111	121	104	119	120	120	120	15	50		47	49		200	50	60
NEOPLASTIC FINDINGS																
B-Tubular Adenoma	1				2											
M-Tubular Carcinoma																

B = benign; M = malignant.
Control groups IA and IB counted together.
+ Study K = common control animals used for two test articles.
* = Gross Lesions only - kidney not routinely examined.
** = No microscopic findings recorded to date.

Note: Search for Renal Tubular Carcinomas revealed no incidence in these studies.

Male Charles River CD-1 Mice
Bio/dynamics, Inc.
KIDNEY

CONTROL DATA

STUDY I.D.	A		B		C		D		E		F		G	
	*	**	*	**	*	**	*	**	*	**	*	**	*	**
Tissue/Finding														
Neoplasm														
No. Examined	57	54	61	51	53	59	60	60	60	60	60	60	60	60
B - Tubular Adenoma		01								02				

*Control Group A	Start	6/78	12/77	12/77	10/78	11/78	11/77	10/77
**Control Group B	Terminate	7/80	4/80	3/80	4/81	4/81	4/80	4/80

Discussion

It can be seen from the above data that the range of historical controls of mouse renal neoplasms from Bio/dynamics is 0 to 3.3 percent. It should be noted that the glyphosate mouse oncogenicity study was conducted by Bio/dynamics between 1980 and 1982. Therefore, the 6 percent incidence of renal tumors in the high-dose group in the glyphosate mouse study exceeds the upper limit of the range of 3.3 percent in the historical

b. Hazleton's Historical Control Data

In a letter dated December 2, 1988 from J.M. Burns of Hazleton to D. Ward of Monsanto, six studies are cited as shown below:

The incidences are for scheduled sacrifices and unscheduled deaths combined.

Study	Type	Init.	Term.	Tubular Cell Carcinoma, Males
1	Dietary	3/80	3/82	2/43
2	Dietary	4/80	4/82	1/100
3	Dietary	9/81	9/83	0/80
4	Dietary	12/79	12/81	0/50
5	Dietary	5/82	5/84	0/60
6	Gavage	8/83	8/85	0/47

Tubular cell carcinomas only were observed.

Discussion

The range of mouse renal neoplasma cited by Hazleton is 0 to 4.7 percent. Therefore, the incidence of 6 percent in the high-dose group of the glyphosate mouse study exceeds the historical controls from Hazleton.

Additional, Monsanto has submitted "representative historical control data" from Hazleton reproduction studies in which renal neoplasia occurred in groups of F_1 generation control mice which were sacrificed after 91 to 105 weeks. These data are shown below:

NEOPLASIA IN CD-1® F_1 MICE - UNTREATED CONTROLS

FINDING	POSITIVE FINDINGS (MALES)	ANIMALS EXAMINED (MALES)
TISSUE NAME--KIDNEY		
TUBULAR CELL ADENOMA		
	1	15
	1	14
POSITIVE TOTALS	2	29
OVERALL TOTALS	2	56
OVERALL PERCENT	3.6	
RANGE OF PERCENTAGES	7	7
TUBULAR CELL CARCINOMA		
	1	15
POSITIVE TOTALS	1	15
OVERALL TOTALS	1	56
OVERALL PERCENT	1.8	
RANGE OF PERCENTAGES	7	7

Discussion

Apparently, this historical control data, which range from 0 to 7.1 percent, are the historical control data cited by Monsanto in their meeting with EPA on November 10, 1988. In a telephone communication on January 30, 1989 to Dr. Ward of Monsanto (314-694-8818), Dr. Ward indicated that Hazleton was unable to provide any additional details (dates of study, supplier, pathologists, etc.) about these particular historical controls. Therefore, in light of this telephone communication, TB concludes that these particular historical controls from F_1 male mice cannot be used to substantiate the Monsanto position.

C. IRDC Historical Control Data

Historical control data from IRDC on the incidences of renal neoplasms in CD-1 male mice in 19 studies of 24 to 25 month duration conducted between 1976 and 1978 are summarized below.

Tumors	No. Tumors	Range	No. Examined
Kidneys			1490
Adenoma	3	0-1.3	
Carcinoma	4	0-1.7	

Discussion

The range of 0 to 1.7 percent for renal neoplasms at IRDC does not exceed the incidence of 6 percent in the high-dose group of the glyphosate mouse study. The submitted historical control data from IRDC did not show the individual study incidences and therefore, is limited in this respect.

D. Spontaneous Renal Neoplasms Observed on 18 Food Color Additive Studies

Monsanto has submitted the incidence of renal neoplasms from 18 food color additive chronic studies with CD-1 mice (supplied to Monsanto by Dr. J.K. Haseman of NIEHS). These data are presented below:

INCIDENCE OF RENAL NEOPLASMS IN CONTROL MALE CD-1 MICE

Study ID[a/]	Testing[b/] Laboratory	Lesion Description	Incidence Group A	Group B
Blue No. 1	IRD	Cortical adenoma	0/60	1/60
Blue No. 2	B/d	Tubular cell adenoma	0/57	1/54
Green No. 3	B/d		0/51	0/53
Green No. 5	HL	Tubular cell adenoma	1/59	0/59
Yellow No. 5	IRD		0/60	0/60

a/A series of chronic bioassays in Charles River CD-1 mice were conducted on 18 food color additives. These studies were sponsored by the Certified Colors Manufacturers Association; the Cosmetic, Toiletries, and Fragrance Association; and the Pharmaceutical Manufacturers Association. Each study utilized 2 concurrent control groups of 60 mice/sex/group. These studies were conducted during the period of 1977 to 1980.

b/Testing laboratories were: International Research and Development Corporation (IRD); Bio/dynamics, Inc. (B/d); Hazleton Laboratories (HL); and Litton Bionetics (LB).

INCIDENCE OF RENTAL NEOPLASMS IN CONTROL MALE CD-1 MICE (Cont'd)

Study ID	Testing Laboratory	Lesion Description	Incidence Group A	Incidence Group B
Yellow No. 6	B/d		0/61	0/60
Yellow No. 10	B/d		0/60	0/60
Orange No. 5	B/d		0/60	0/60
Orange No. 17	B/d	Tubular cell adenoma	0/60	2/60
Red No. 3	IRD		0/60	0/60
Red No. 6	IRD		0/60	0/60
Red No. 9	LB	Tubular cell adenoma	0/59	2/60
Red No. 9		Tubular cell adenocarcinoma	1/59	0/60
		Cholesterol granuloma	1/59	0/60
Red No. 19	B/d		0/54	0/57
Red No. 21	IRD	Adenoma (N.O.S.)	1/60	0/60
Red No. 27	LB	Tubular cell adenoma	1/60	0/59
		Hemagiosarcoma	1/60	0/59
Red No. 30	HL		0/60	0/58
Red No. 33	IRD	Tubular cell adenoma	1/60	0/60
		Cortical carcinoma	1/60	0/60
Red No. 36	LB		0/60	0/60

Discussion

The incidence of renal tubular neoplasms ranged from 0 to 3.3 percent. It should be noted that the 3.3 percent incidence (2/60) of tubular cell adenoma in Orange No. 17 from Bio/dynamics was previously reported by Monsanto as historical

control data by Bio/dynamics and does not represent additional findings. The incidence of 3.3 percent (2/60) for renal tubular cell adenoma in Red No. 9 from Litton Bionetics was not previously reported and is considered new data.

E. Historical Control Data in CD-1 Mice From The Institute of Environmental Toxicology (Tokyo, Japan).

The incidence of renal neoplasms from male CD-1 mice was 6/891 (0.67%). In a telephone communication on January 30, 1989 with Dr. Ward of Monsanto, Dr. Ward indicated that for individual studies the incidence of renal neoplasms ranged from 0 to 1.4 percent (1/70). The range of 0 to 1.4 percent of renal neoplasms is comparable to the incidences observed at other laboratories.

Attachments

R:53487:Dykstra:C.Disk:KENCO:2/6/89:CT:VO:CT
R:57213:Dykstra:C.Disk:KENCO:5/7/89:rw:vo:jh:dg

Attachment C

CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Glyphosate - Meeting with Monsanto Regarding EPA's Requirement that the Mouse Oncogenicity Study be Repeated

TOX Chem No.: 661A

FROM: Edwin Budd, Section Head
Toxicology Branch - Insecticide, Rodenticide Support
Health Effects Division (TS-769C)

11/15/88

TO: Robert J. Taylor, PM 25
Fungicide-Herbicide Branch
Registration Division (TS-767C)

On November 10, 1988, EPA staff met with representatives from Monsanto Company to discuss the Agency's requirement that the mouse oncogenicity study on glyphosate be repeated. The meeting was requested by Monsanto in a letter dated October 5, 1988 (attached), which also briefly outlined some of Monsanto's rationale supporting their contention that "there is no relevant scientific or regulatory justification for repeating the glyphosate mouse oncogenicity study." The following persons attended the meeting:

EPA Staff	Monsanto Company
Anne Lindsay	Chester Dickerson, Jr.
Frank Sanders	Kevin Cannon
Robert J. Taylor	Dennis Ward
William Burnam	
Edwin Budd	

Dennis Ward initiated the meeting by recounting pertinent findings in the rat and mouse oncogenicity studies on glyphosate, recalling prior EPA and SAP assessments of the available data,

presenting Monsanto's rationale and conclusions regarding the totality of the available data relating to the oncogenic potential of glyphosate, and reiterating Monsanto's contention that there is no scientific or regulatory justification for repeating the mouse study. Highlights of his presentation are itemized in a document handed out at the meeting (attached).

In response to Dennis Ward's presentation, Edwin Budd said he did not recall having previously seen historical control data on kidney tumors in mice with a range in individual studies of 0 to 7.1 percent. Dr. Ward stated that he believed this information (on individual studies) had been submitted to EPA some time ago. Inasmuch as a major reason for EPA's concern regarding the kidney tumors in the mouse study was the belief that such tumors are quite rare in mice, the meeting participants agreed that it would be appropriate to again consider relevant historical control data on the matter, and particularly the findings in individual studies. Toward this end, Monsanto agreed to locate and again submit to the Agency as soon as possible what they believe to be pertinent historical control data on individual studies. EPA, in turn, agreed to evaluate the usefulness and content of the data and utilize it, as appropriate, in a reconsideration of EPA's prior requirement that the mouse study be repeated.

Attachments

cc: William Burnam
Judith Hauswirth
William Dykstra

CASWELL FILE

Monsanto

Monsanto Company
1101 17th Street, N.W.
Washington, D.C. 20036
Phone: (202) 452-8880

October 5, 1988

Director
Registration Division (TS767C)
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Crystal Mall #2, Room 1116
Arlington, Virginia 22202

Attention: Mr. Edwin F. Tinsworth

Subject: Glyphosate Registration Standard: Request for Meeting with EPA

Dear Sir:

On September 7, 1988 the Agency submitted to Monsanto a scientific review and evaluation of our November 7, 1986 response to the glyphosate Registration Standard issued in August, 1986. Part of that response included a request for a waiver of the requirement to repeat the glyphosate mouse oncogenicity study. The Agency's letter indicates that you have decided not to concur with our waiver request and have asked for Monsanto "to conduct a specially designed study for the specific purpose of clarifying certain unresolved questions relating to the potential oncogenicity of glyphosate."

Monsanto's position regarding this requirement has been, and remains to be, that there is no scientific justification for repeating the mouse oncogenicity study. The results of the current mouse oncogenicity study have been reviewed by numerous toxicologists and pathologists from both the private sector and universities, as well as by the FIFRA Scientific Advisory Panel. The unanimous conclusion of all of these experts was that this study did not provide evidence that glyphosate was oncogenic to mice. The study was conducted at dosages far in excess of those required by the Agency's MTD Position Paper, and the response in question (a spurious, not statistically significant increase in a benign kidney tumor) occurred in only three male mice at a dosage level of 30,000 ppm glyphosate in the diet.

Repeating this study will not provide any new information which would be useful for regulatory purposes. Monsanto has analyzed the effects of dosage levels, tumor dose response and number of animals tested on the hypothetical Q1* which would result. In the twenty cases we analyzed, which covered dosage levels and group sizes including those suggested by the Agency for a repeat study, it was clear that there was no significant difference in the calculated Q1* values. This was true even when the hypothetical tumor response rate was double that observed in the original mouse study. Multiplying the very low calculated Q1*'s by the low level of potential human exposure to glyphosate (1) results in potential levels of human risk far less than 1 x 10 -6.

The low values obtained for the calculated Q1*'s and their insensitivity to percent tumor response and number of animals tested underscore the lack of value in conducting another study to characterize glyphosate in EPA's ranking scheme. Monsanto believes it may be more useful to use the results of the ongoing repeat rat chronic feeding/oncogenicity study to determine whether any change from the current "D" classification is in order.

Based upon the above discussion, Monsanto contends that there is no relevant scientific or regulatory justification for repeating the glyphosate mouse oncogenicity study. We feel that to do so would not be an appropriate use of either the Agency's or Monsanto's resources. I would like to have the opportunity to meet with you to review this issue in further detail, and have asked Dr. Kevin Cannon to contact Mr. Robert Taylor to schedule a mutually agreeable date. I would like to suggest October 19, 1988 as a possibility.

Thank you for your attention to this issue, and if you should have any questions regarding this request, please contact either Dr. Kevin Cannon in our Washington office or me.

Sincerely,

George B. Fuller, Ph.D.
United States and International
Registration Director

References

1. An excellent review of potential human risks from exposure to glyphosate under hypothetical "worst case" scenarios can be found in a document prepared by K.S. Crump and Associates for the state of Washington, Department of Natural Resources: Shipp, A.M. et al. (1986). Worst Case Analysis Study On Forest Plantation Herbicide Use. See specifically Chapter 5. Risk Assessment for Glyphosate, pp. 132-140.

/bj
cc: K.F. Cannon

handed out by Monsanto at 11/10/8 meeting

Meeting with EPA, November 10, 1988 on:

CASWELL FILE

GLYPHOSATE -- ISSUES RELATED TO ONCOGENICITY

1. Rat study -- No treatment related tumors; however, an MTD was not achieved. Replacement study in progress.

2. Mouse study -- Ongoing disagreement over interpretation of kidney tumors in male mice:

Dose Level (ppm)	0	1000	5000	30,000
Tubular adenoma	1	0	1	3
Animals examined	49	49	50	50

3. Monsanto conclusion -- These tumors are not treatment related:

- lack of significance in pair-wise comparison test;
- lack of significance in age-adjusted trend test;
- high dose incidence is within historical control ranges (0-7.1%);
- mechanistic considerations: glyphosate is not metabolized by rodents and is not genotoxic; promotional mechanism is unlikely due to lack of cytotoxicity, inflammatory responses, or preneoplastic changes in target organ;
- unanimous conclusion of third party pathologists that these tumors are not treatment related;
- FAO/WHO has concluded "... no evidence of carcinogenicity".

4. S.A.P. conclusion - Equivocal, Category D

   - small number of tumors at HDT which appears to have exceeded the MTD

   - "... no oncogenic effect of Glyphosate is demonstrated using concurrent controls."

   - "... the level of concern raised by historical control data was not great enough to displace putting primary emphasis on the concurrent controls."

5. Toxicology Branch conclusion - "... the oncogenic potential of glyphosate could not be determined from existing data and proposes that the study be repeated in order to clarify these equivocal findings."

6. Monsanto continues to believe that a weight-of-evidence evaluation strongly supports conclusion that glyphosate is not oncogenic in the mouse. Results of the ongoing chronic rat study will answer questions about oncogenic potential of glyphosate.

7. Repeating the mouse study is unlikely to "... clarify these equivocal findings." Answering this academic question would require the expenditure of significant resources, the wasting of hundreds of additional laboratory animals, and would tie-up valuable laboratory space.

8. Repeating the mouse study would have no impact on the regulatory management of glyphosate, regardless of study outcome. Estimates of risk could only decrease (refer to attached tables).

Table 1. Effect of group size (n) on linearized multistage model slope (q_1^*) with a constant tumor response rate.

	Tumor Incidence (%)				
Group Size	0	1000	5000	30,000	q_1^* $(mg/kg/d)^{-1}$
n = 50	2	0	2	6	3.2×10^{-4}
n = 100	2	0	2	6	2.6×10^{-4}
n = 200	2	0	2	6	2.2×10^{-4}

Dose levels of 0, 1000, 5000 and 30,000 ppm in diet correspond to human equivalent doses $(BW)^{0.67}$ of 0, 12.5, 64.6, and 384 mg/kg/d.

Table 2. Effect of group size (n) and hypothetical tumor response rate on linearized multistage model slope (q_1^*).

Group Size	Tumor Incidence (%) 0	7500	15,000	30,000 ppm	q_1^* $(mg/kg/d)^{-1}$
n = 50	2	0	2	6	1.9×10^{-4}
n = 200	2	0	2	6	7.0×10^{-5}
n = 200	0	0	0	12	2.9×10^{-5}
n = 200	0	0	4	12	1.5×10^{-4}

Dose levels of 0, 7500, 15,000 and 30,000 ppm in diet correspond to human equivalent doses $(BW)^{0.67}$ of 0, 96.0, 192, and 384 mg/kg/d.

Monsanto

Monsanto Company
1101 - 17th Street N.W.
Washington, D.C. 20036
(202) 452-8860

Chester T. Dickerson, Jr., Ph.D.
Director, Agricultural Affairs

Monsanto

Monsanto Company
1101 17th Street N.W.
Suite 604
Washington, D.C. 20036
(202) 452-8880

Kevin F. Cannon, Ph.D.
Manager, Agricultural Affairs

Monsanto

Monsanto Agricultural Company
800 N. Lindbergh Boulevard
St. Louis, Missouri 63167
(314) 694-8818

Dennis P. Ward, Ph.D.
Diplomate, American Board of Toxicology
Product Toxicology Specialist

CASWELL FILE 9-697

[redacted]

Attachment

Monsanto

Monsanto Company
1101 17th Street, N.W.
Washington, D.C. 20036
Phone: (202) 452-8880

December 12, 1988

Director
Registration Division (TS-767C)
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Crystal Mall #2, 726
Arlington, VA 22202

Attention: Mr. Robert J. Taylor
Product Manager (25)

Subject: Response to EPA request for historical control data.
Re: Glyphosate chronic feeding study in mice.
EPA MRID No. 00130406
Meeting held with Registration and Health Effects Divisions on November 10, 1988

Dear Sir:

On November 10, 1988, a meeting was held between representatives of the EPA and Monsanto to discuss the Agency's request for a second glyphosate oncogenicity study in mice. This request is based on an Agency finding that the existing mouse study provides equivocal data with regard to glyphosate's oncogenic potential. Monsanto, on the other hand, has interpreted the results of this study to indicate that glyphosate is not oncogenic in the mouse. Persons in attendance at this meeting were E. Budd, W. Burnam, A. Lindsay, F. Sanders and R. Taylor, representing the EPA, and K. Cannon, C. Dickerson and D. Ward, representing Monsanto. The bases for our respective interpretations were discussed. At the conclusion of our meeting, Drs. Budd and Burnam agreed to reconsider their interpretation of the the existing study and requested that Monsanto provide additional historical control data to aid in their evaluation. The requested data are attached as Appendices A through E.

The existing glyphosate oncogenicity study in mice (Study No. BD-77-420/77-2061; EPA MRID No. 00130406) was conducted between 1980 and 1982 at Bio/dynamics, Inc. Glyphosate was administered to Charles River CD-1 mice for 24-25 months at dietary concentrations of 0, 1000, 5000 and 30,000 ppm. The high dose level of 30,000 ppm is considered to have met, and perhaps exceeded an MTD. On this study a small difference in control and high dose male kidney tumor incidence was observed. Whether or not this difference in tumor rates is attributable to glyphosate administration has been a point of disagreement between Monsanto and the Agency. The following table summarizes the occurrence of renal tubular cell neoplasms in male mice on this study:

	Tumor Incidence			
Dose Level (ppm)	0	1000	5000	30,000
Animals examined	49	49	50	50
Tubular adenoma	1 (2)	0 (0)	1 (2)	3 (6)

Percent incidence in parentheses ().

The EPA Toxicology Branch has considered the relationship of glyphosate administration to the occurrence of kidney tumors in high dose males to be equivocal. Monsanto has concluded that there is no relationship between the two. We appreciate the Agency's willingness to re-examine this issue and respectfully request that EPA scientists consider the following points in their evaluation:

i. The absence of significance at the $p \leq 0.05$ level in the pair-wise comparison and linear trend analyses (i.e. Fisher's Exact Test and Cochran-Armitage Trend Test) is a strong indication that the occurrences of these neoplasms reflect random distribution.

ii. These renal neoplasms were detected only in males sacrificed at termination of the study. Had these been chemically induced neoplasms one might have expected to observe a reduction in the latency period.

iii. The observed renal neoplasms all occurred unilaterally with no evidence of multiplicity of form.

iv. Tubular cell neoplasms were not detected in any female mice on this study although they received significantly more glyphosate (21%) than males at the high dose level (on a mg/kg/day basis).

v. Compound related nephrotoxicity, including preneoplastic changes, were not present on this study. Likewise, renal toxicity was not observed at an even higher dose level (i.e. 50,000 ppm) employed on the subchronic study (Study No. BD-77-419; EPA MRID No. 242799). We are unaware of any instances in which a renal carcinogen was administered at dose levels sufficient to induce tumors without also inducing tubular toxicity and hyperplasia.

vi. An extensive battery of genotoxicity assays have been performed with glyphosate (i.e. Ames/Salmonella, host mediated assay, E. coli reverse mutation, CHO/HGPRT, in vivo cytogenetics, dominant lethal and hepatocyte UDS). Glyphosate was found to be uniformly negative in these assays and is therefore, considered to be nongenotoxic.

vii. The metabolism of glyphosate has been extensively studied in rodents. Glyphosate is not metabolized by rodents (or presumably other mammalian species) and has been found to be quantitatively excreted unchanged following oral administration. If one assumed that glyphosate was responsible for the kidney tumors observed in male mice, then metabolic activation of the molecule must not have been a requisite step for tumor formation. The uniformly negative short-term test results are not consistent with glyphosate being a direct acting genotoxic agent. It is highly improbable that the aforementioned genotoxicity assays would have failed to detect a direct acting carcinogen. It is equally unlikely that these tumors arose through a promotional mechanism because of the absence of glyphosate induced cytotoxic or preneoplastic changes in the affected organ.

viii. The detailed information on historical control data requested by Drs. Budd and Burnam can be found in the attached appendices. Appendices A, B and C contain study-by-study incidences of renal tumors in untreated male CD-1 mice on two year studies conducted at Bio/dynamics, Hazleton and IRDC, respectively.

In studies conducted at Bio/dynamics, Inc., spontaneous renal tubular cell neoplasms have been observed at rates ranging from 0 to 3.3%. On chronic studies conducted at Hazleton Laboratories America, spontaneous renal neoplasms have been observed at rates ranging from 0 to 4.7%. In addition, a limited amount of data is available from four Hazleton reproduction studies in which smaller groups of F1 generation animals were maintained on study for 91 to 105 weeks. Spontaneous renal tubular cell neoplasms were observed at rates ranging from 0 to 7%. The utility of these four studies is perhaps limited by the small number of animals examined. On studies conducted at International Research and Development Corp., spontaneous renal neoplasms were observed at rates ranging from 0 to 1.7%. It should be noted that it is not possible to determine individual study incidences for combined kidney adenomas and carcinomas in the IRDC database, which would have been helpful.

We are also enclosing for Agency review in Appendices D and E, data from 18 food color additive chronic studies with CD-1 mice (supplied courtesy of Dr. J. K. Haseman of NIEHS) and data from 11 chronic studies with CD-1 mice conducted at The Institute of Environmental Toxicology (Tokyo, Japan). On the food color additive studies, spontaneous renal tubular cell neoplasms were observed at rates ranging from 0 to 3.3% (Appendix D). [Note: There is some overlap of Bio/dynamics studies included in this appendix and in Appendix A.] On studies conducted at The Institute of Environmental Toxicology, spontaneous renal neoplasms were observed at rates ranging from 0 to 1.4% (Appendix E).

Review of these historical control data indicates that renal tubular cell neoplasms are not 'common' tumors in male CD-1 mice. They do however, occur sporadically on two year studies and with considerable variability from study to study. The 6% incidence observed in the glyphosate high dose group is higher that the 4.7% incidence observed on a comparable study conducted at Hazleton, but only slightly higher (i.e. by only one tumor).

From review of other studies that Monsanto has conducted with CD-1 mice, it is apparent that these tumors appear with some regularity on chronic bioassays and always in aged animals. Furthermore, clusters of these tumors appear on some studies but not others. This may be attributable to genetic or environmental factors affecting whole study populations of animals. On some chronic studies none of these tumors were observed, yet on other studies as many as nine have been detected. As examples, renal tubular cell adenomas and/or carcinomas have been observed at the following rates on two Monsanto contracted studies:

	Tumor Response Rate [a]			
	Control	Low-dose	Mid-dose	High-dose
Study I	0/80	2/80	1/80	2/80
Study II	2/50	1/50	3/50	3/50

a - Number of males with kidney tumors/number of males examined.

Study I - A two year study conducted with butachlor at Hazleton Laboratories America between 1981 and 1983 with Charles River CD-1 mice.

Study II - A two year study conducted with MON 097 at Pharmacopathics Research between 1980 and 1982 with Charles River CD-1 mice.

On neither of these studies is there reason to conclude that the observed kidney tumors are attributable to administration of the test compounds. The distribution of tumors between groups on these two studies is clearly random. Both studies provide examples of how these tumors tend to cluster in subpopulations of animals. The total number of renal tumors observed on these studies is comparable to the number observed on the glyphosate study. The incidence of renal tubular cell neoplasms in two of the study groups is identical to the glyphosate high dose group incidence (i.e. 6%).

In summary, the 6% incidence of renal tumors in high dose males is unusual. However, no other data supports a conclusion of these tumors being treatment related. It is the judgement of Monsanto scientists that the weight-of-evidence strongly supports a conclusion that glyphosate is not oncogenic in the mouse. Numerous third party experts in pathology and toxicology have evaluated this study and unanimously concluded that glyphosate is not oncogenic in the mouse (the written opinions of these experts were previously submitted to the Agency). EPA concordance with this interpretation would of course obviate the need for a second mouse study. We respectfully request that EPA scientists consider all of these factors in re-evaluation of this study. In the event that a second study remains necessary, we request that initiation of the allotted 50 month period (re: EPA letter dated September 7, 1988) be delayed pending completion of this re-evaluation. Furthermore, we consider the information contained herein to be confidential and request that it be treated as such. Thank you.

We will of course, be glad to provide any additional information needed by the Agency for resolution of this issue. Such requests can be made through Dr. Kevin Cannon at our Washington Office (202-452-8880) or directly through me (314-694-8818).

Sincerely,

Dennis P. Ward, Ph.D., DABT
Senior Toxicology Specialist

/jjs

cc: K.F. Cannon, Monsanto WA,D.C.
C.T. Dickerson, Monsanto WA,D.C.

Attachments: Appendices A-E

APPENDIX A:

Bio/dynamics Historical Control Data

CD-1 (COBS) Mice

Bio/dynamics, Inc.

SELECTED ORGAN - Kidneys
CONTROL DATA

Long-Term Carcinogenicity Studies
Historical Control Data
CD-1 (COBS) Mice

TABLE OF CONTENTS

CONTROL DATA FOR CD-1, COBS (ICR Derived) MICE
Study Design & General Information

Study Code	Duration (Months)	Initial No. on Test (#/sex/grp)	Supplier	Age at Initiation	Date of Initiation	Date of Termination	Clin Lab Intervals (Months)	Interim Sacrifices (Months)
STUDY A*	22 M 23 F	60	Charles River Wilmington, MA	42 days	5-16-78	2-22-80 M 4-15-80 F	3,6,12,18	None
STUDY B*	20 M 23 F	60	Charles River Wilmington, MA	42 days	5-16-78	12-27-79 M 3-31-80 F	3,6,12,18	None
STUDY C*	25 M 25 F	60	Charles River Wilmington, MA	43 days	5-11-78	5-16-80	3,6,12,18	None
STUDY D*	22 M 25 F	60	Charles River Wilmington, MA	44-51 days	3-17-78	1-15-80 M 4-11-80 F	3,6,12,18	None
STUDY E*	23 M 25 F	60	Charles River Wilmington, MA	47-54 days	3-20-78	1-31-80 M 3-28-80 F	3,6,12,18	None
STUDY F*	24 M 23 F	60	Charles River Wilmington, MA	45-52 days	3-31-78	3-14-80 M 2-13-80 F	3,6,12,18	None
STUDY G*	24 M 24 F	60	Charles River Wilmington, MA	43-50 days	3-17-78	2-25-80 M 2-20-80 F	3,6,12,18	None
STUDY H	22	100	Charles River Wilmington, MA	42 days	10-8-80	8-2-82	None	None
STUDY I	19	50	Charles River Wilmington, MA	53 days	4-14-78	10-18-79	12, Term	None
STUDY J	24	75	Charles River Wilmington, MA	52-59 days	12-3-76	12-10-78	24	None
STUDY K**	18	70	Charles River Wilmington, MA	48 days	6-21-76	1-3-78	18 (Diffs)	6,12
STUDY L	24	50	Charles River Portage, MI	40 days	3-31-80	3-14-82	12,18,24	None
STUDY M	24	50	Charles River Portage, MI	6 weeks	2-12-80	2-18-82	12,18,24	None
STUDY N***	25	100	Charles River Portage, MI	40 days	10-10-80	11-5-82	3,6,12,18,24 & Monthly	None

*This study had two control groups (1A and 1B) with 60 animals per sex in each group.
**This study had two control groups (1A and 1B) with 70 animals per sex in each group.
***This study had two control groups (1A and 1B) with 100 animals per sex in each group.

10/7/85

CONTROL DATA FOR CD-1, COBS (ICR Derived) MICE
Study Design & General Information

Study Code	Duration (Months)	Initial No. on Test (#/sex/grp)	Supplier	Age at Initiation	Date of Initiation	Date of Termination	Clin Lab Intervals (Months)	Interim Sacrifices (Months)
STUDY O	20 M 20 F	50	Charles River Portage, MI	41 days	3-30-82	11-3-83	6, 18	None
STUDY P	19 M 19 F	60	Charles River Kingston, NY	41 days	7-30-82	2-9-84	18	None

CD-1 COBS (ICR Derived) Mice

Bio/dynamics, Inc.

MALES - KIDNEYS

C O N T R O L D A T A

STUDY I.D.	A	B	C	D	E	F	G	H*	I	J**	K †	L	M**	N	O	P
Tissue/Finding																
# Examined	111	121	104	119	120	120	120	15	50		47	49		200	50	60
NEOPLASTIC FINDINGS																
B-tubular adenoma	1				2											
M-tubular carcinoma																

B benign M malignant
Control groups IA and IB counted together.
† Study K = common control animals used for 2 test articles.
* = Gross Lesions only - kidney not routinely examined.
** = No microscopic findings recorded to date.

Note: Search for Renal Tubular Carcinomas revealed no incidence in these studies.

Male Charles River CD-1 Mice
Bio/dynamics, Inc.

KIDNEY

CONTROL DATA

STUDY I.D.	A		B		C		D		E		F		G	
	*	**	*	**	*	**	*	**	*	**	*	**	*	**
Tissue/Finding														
Neoplasm														
# Examined	57	54	61	60	51	53	59	60	60	60	60	60	60	60
B - tubular adenoma		01								02				

* Control Group A	Start	6/78	12/77	12/77	10/78	11/78	11/77	10/77
** Control Group B	Terminate	7/80	4/80	3/80	4/81	4/81	4/80	4/80

CD-1 COBS (ICR Derived) Mice

Bio/dynamics, Inc.

FEMALES - KIDNEYS

CONTROL DATA

STUDY I.D.	A	B	C	D	E	F	G	H*	I	J**	K †	L	M**	N	O	P
Tissue/Finding																
# Examined	107	116	102	115	119	120	120	18	50		60	50		200	47	60
NEOPLASTIC FINDINGS																
B-tubular adenoma																
M-tubular carcinoma																

B benign M malignant
Control groups IA and IB counted together.
† Study K = common control animals used for 2 test articles.
* = Gross Lesions only - kidney not routinely examined.
** = No microscopic findings recorded to date.

Note: Search for Renal Tubular Adenomas and Carcinomas revealed no incidence in these studies.

APPENDIX B:

Hazleton Historical Control Data

HAZLETON
LABORATORIES AMERICA, INC.
9200 LEESBURG TURNPIKE, VIENNA, VIRGINIA 22180 U.S.A.

December 2, 1988

Dennis Ward, Ph.D C2SE
Monsanto Company
800 North Lindbergh Blvd.
St. Louis, MO 63167

Dear Dr. Ward:

Below are the incidences of spontaneous renal tumors (all types) in CD-1 male mice which you requested. All studies lasted 2 years and have been finalized. None of this data was included in the Hazleton historical data publications.

The incidences are for scheduled sacrifices and unscheduled deaths combined.

Study	Type	Init.	Term.	Tubular Cell Carcinoma, Males
1	Dietary	3/80	3/82	2/ 43
2	Dietary	4/80	4/82	1/100
3	Dietary	9/81	9/83	0/ 80
4	Dietary	12/79	12/81	0/ 50
5	Dietary	5/82	5/84	0/ 60
6	Gavage	8/83	8/85	0/ 47

As you can see, tubular cell carcinomas were the only kidney neoplasms observed.

If I may be of further assistance, please do not hesitate to call me.

Sincerely,

John M. Burns

JOHN M. BURNS, M.S., D.V.M.
Life Sciences Division

JMB:clw

HAZLETON
LABORATORIES AMERICA, INC.
9200 LEESBURG TURNPIKE, VIENNA, VIRGINIA 22180, U.S.A.

REPRESENTATIVE HISTORICAL CONTROL DATA

PART I:	RODENT LONGEVITY
PART II:	NEOPLASIA IN SPRAGUE-DAWLEY RATS
PART III:	NEOPLASIA IN UNTREATED B6C3F1 MICE
PART IV:	NEOPLASIA IN B6C3F1 CONTROL MICE TREATED WITH CORN OIL IN THE DIET
PART V:	NEOPLASIA IN B6C3F1 CONTROL MICE TREATED WITH CORN OIL ADMINISTERED BY GAVAGE
PART VI:	NEOPLASIA IN B6C3F1 CONTROL MICE TREATED WITH CARBOXYMETHYL-CELLULOSE ADMINISTERED BY GAVAGE
PART VII:	NEOPLASIA IN UNTREATED CD-1® MICE
PART VIII:	NEOPLASIA IN UNTREATED CD-1® F1 MICE
PART IX:	NEOPLASIA IN CD-1® CONTROL MICE TREATED WITH DISTILLED WATER ADMINISTERED BY GAVAGE
PART X:	NEOPLASIA IN CD-1® CONTROL MICE TREATED WITH 0.5% TRAGACANTH IN DISTILLED WATER ADMINISTERED BY GAVAGE
PART XI:	HEMATOLOGY REFERENCE RANGES
PART XII:	CLINICAL CHEMISTRY REFERENCE RANGES

NOTE: Historical control data generated in-house at Hazleton Laboratories America, Inc.

updated 7/1/83

REPRESENTATIVE HISTORICAL CONTROL DATA

PART VII

NEOPLASIA IN UNTREATED CD-1® MICE

HAZLETON LABORATORIES AMERICA, INC.
SUMMARY OF NEOPLASIA IN UNTREATED CONTROL CD-1® MICE

THE FINDINGS PRESENTED IN THIS SUMMARY ARE FROM UNTREATED CONTROL MICE SACRIFICED AFTER 91 TO 105 WEEKS.

THE TERM 'POSITIVE TOTALS' REPRESENTS THE TOTAL NUMBER OF POSITIVE FINDINGS FROM STUDIES WHERE THERE WERE ONE OR MORE OCCURRENCES OF THE INDICATED NEOPLASM IN EACH SEX. THE DATA FROM THESE STUDIES, INCLUDING THE NUMBER OF TISSUES EXAMINED, ARE PRESENTED.

THE TERM 'OVERALL TOTALS' REPEATS THE TOTAL NUMBER OF POSITIVE FINDINGS AND ALSO PRESENTS THE TOTAL NUMBER OF TISSUES OBSERVED FROM ALL QUALIFYING STUDIES, THAT IS, THOSE STUDIES WITH POSITIVE AS WELL AS NEGATIVE FINDINGS.

WHEN POSITIVE FINDINGS ARE LISTED FOR TISSUE MASS, OTHER LESIONS, MULTIPLE ORGANS, OR OTHER NON-PROTOCOL TISSUES, THE TOTAL NUMBER OF TISSUES EXAMINED REPRESENTS THE TOTAL NUMBER OF ANIMALS EXAMINED AT THAT INTERVAL OR THE TOTAL NUMBER OF ANIMALS ON STUDY, AS APPROPRIATE.

'OVERALL PERCENT' IS THEN CALCULATED USING THE 'OVERALL TOTALS' FIGURE.

THE COMPUTER ESTABLISHES 'RANGE OF PERCENTAGES' FROM THE DATA COMPRISING 'POSITIVE TOTALS'.

NEOPLASIA IN CD-1® MICE-UNTREATED CONTROLS

FINDING	POSITIVE FINDINGS (MALES)	ANIMALS EXAMINED (MALES)	POSITIVE FINDINGS (FEMALES)	ANIMALS EXAMINED (FEMALES
*** TISSUE NAME--KIDNEY ***				
CARCINOMA				
	1	10	0	10
POSITIVE TOTALS--	1	10	0	10
OVERALL TOTALS---	1	284	0	294
OVERALL PERCENT--	0.4		0.0	
RANGE OF PERCENTAGES--	10 --	10	0 --	0
*** TISSUE NAME--LIP ***				
PAPILLOMA				
	0	10	1	10
POSITIVE TOTALS--	0	10	1	10
OVERALL TOTALS---	0	10	1	10
OVERALL PERCENT--	0.0		10.0	
RANGE OF PERCENTAGES--	0 --	0	10 --	10

REPRESENTATIVE HISTORICAL CONTROL DATA

PART VIII

NEOPLASIA IN UNTREATED CD-1® F1 MICE

HAZLETON LABORATORIES AMERICA, INC.
SUMMARY OF NEOPLASIA IN UNTREATED CONTROL CD-1® F1 MICE

THE FINDINGS PRESENTED IN THIS SUMMARY ARE FROM UNTREATED F1 GENERATION CONTROL MICE SACRIFICED AFTER 91 TO 105 WEEKS.

THE TERM 'POSITIVE TOTALS' REPRESENTS THE TOTAL NUMBER OF POSITIVE FINDINGS FROM STUDIES WHERE THERE WERE ONE OR MORE OCCURRENCES OF THE INDICATED NEOPLASM IN EACH SEX. THE DATA FROM THESE STUDIES, INCLUDING THE NUMBER OF TISSUES EXAMINED, ARE PRESENTED.

THE TERM 'OVERALL TOTALS' REPEATS THE TOTAL NUMBER OF POSITIVE FINDINGS AND ALSO PRESENTS THE TOTAL NUMBER OF TISSUES OBSERVED FROM ALL QUALIFYING STUDIES, THAT IS, THOSE STUDIES WITH POSITIVE AS WELL AS NEGATIVE FINDINGS.

WHEN POSITIVE FINDINGS ARE LISTED FOR TISSUE MASS, OTHER LESIONS, MULTIPLE ORGANS, OR OTHER NON-PROTOCOL TISSUES, THE TOTAL NUMBER OF TISSUES EXAMINED REPRESENTS THE TOTAL NUMBER OF ANIMALS EXAMINED AT THAT INTERVAL OR THE TOTAL NUMBER OF ANIMALS ON STUDY, AS APPROPRIATE.

WHERE INDIVIDUAL STUDY DATA ARE FOLLOWED BY THE SUPER-SCRIPT 'A', THE NUMBER PRESENTED REPRESENTS THE NUMBER OF ANIMALS SACRIFICED AT TERMINATION RATHER THAN THE NUMBER OF TISSUES EXAMINED.

'OVERALL PERCENT' IS THEN CALCULATED USING THE 'OVERALL TOTALS' FIGURE.

THE COMPUTER ESTABLISHES 'RANGE OF PERCENTAGES' FROM THE DATA COMPRISING 'POSITIVE TOTALS'.

NEOPLASIA IN CD-1® F1 MICE-UNTREATED CONTROLS

FINDING	POSITIVE FINDINGS (MALES)	ANIMALS EXAMINED (MALES)	POSITIVE FINDINGS (FEMALES)	ANIMALS EXAMINED (FEMALES)
*** TISSUE NAME--KIDNEY ***				
TUBULAR CELL ADENOMA				
	1	15	0	15
	1	14	0	26
POSITIVE TOTALS--	2	29	0	41
OVERALL TOTALS---	2	56	0	81
OVERALL PERCENT--	3.6		0.0	
RANGE OF PERCENTAGES--	7 --	7	0 --	0
TUBULAR CELL CARCINOMA				
	1	15	0	15
POSITIVE TOTALS--	1	15	0	15
OVERALL TOTALS---	1	56	0	81
OVERALL PERCENT--	1.8		0.0	
RANGE OF PERCENTAGES--	7 --	7	0 --	0
*** TISSUE NAME--LIVER ***				
HEMANGIOSARCOMA				
	0	15	2	15
POSITIVE TOTALS--	0	15	2	15
OVERALL TOTALS---	0	75	2	100
OVERALL PERCENT--	0.0		2.0	
RANGE OF PERCENTAGES--	0 --	0	13 --	13

APPENDIX C:

IRDC Historical Control Data

1971- 1978

Tumor Incidences in Control Mice (CD-1)

Tumor incidences in control mice from long term studies at IRDC are given in the appended tables. In view of the variable length of such studies the incidences are given in three tables:

-18 Month Studies: Incidences from 6 studies conducted between 1971 and 1975 (Number of animals represented: 340 males, 300 females).

-20-22 Month Studies: Incidences from 7 studies conducted between 1973 and 1978 (Number of animals represented: 473 males, 420 females).

-24-25 Month Studies: Incidences from 19 studies conducted between 1976 and 1978 (Number of animals represented: 1490 males, 1490 females).

It should be noted that certain single cases of tumor were omitted, with corresponding corrections to the denominator groups, where classification for the tables presented problems (eg; where no tissue site was specified). As not all animals for all tissues were examined in all studies, (particularly in the case of lesions in unusual sites), the percentages are derived from the number of animals with the tumor divided by the total number of animals in the study groups ("animals at risk").

Location and type of tumors	MALES			FEMALES		
	Total Number of Animals with Tumors	Total Mean % Incidence	Range of % Incidence in Studies	Total Number of Animals with Tumors	Total Mean % Incidence	Range of % Incidence in Studies
Testes						
interstitial cell adenoma	11	0.7	0- 4.0			
epididymis:hemangioma	1	0.1	0- 0.4			
Ovaries						
papillary adenoma				4	0.3	0- 3.3
thecal cell adenoma				2	0.1	0- 1.7
cystadenoma/adenoma				21	1.4	0-11.7
granulosa cell adenoma				4	0.3	0- 1.7
carcinoma				1	0.1	0- 1.7
stromal sarcoma				1	0.1	0- 1.0
dysgerminoma				2	0.1	0- 1.7
neuroblastoma				1	0.1	0- 1.7
hemangioma				4	0.3	0- 1.7
Spleen						
hemangioma	6	0.4	0- 2.0	6	0.4	0- 3.3
hemangiosarcoma	10	0.7	0- 2.0	9	0.6	0- 3.3
Liver						
hepatocellular adenoma	34	2.3	0-26.7	13	0.9	0- 6.0
hepatocellular carcinoma	125	8.4	1.0-26.0	15	1.0	0-10.0
hemangioma	4	0.3	0- 2.0	5	0.3	0- 4.0
hemangiosarcoma	4	0.3	0- 3.3	8	0.5	0- 3.3
angiosarcoma	0	0	--	1	0.1	0- 1.0
liposarcoma	0	0	--	1	0.1	0- 1.7
cholangioma	1	0.1	0- 1.7	1	0.1	0- 1.0
Kidneys						
adenoma	3	0.2	0- 1.3	3	0.2	0- 1.7
carcinoma	4	0.3	0- 1.7	0	0	--
Uterus/Cervix						
leiomyoma				27	1.8	0- 6.0
leiomyosarcoma				29	1.9	0- 9.0
polyp				71	4.8	0-12.0
adenoma				2	0.1	0- 1.7
follicular adenoma				1	0.1	0- 1.7

24-25 MONTH STUDIES	MALES			FEMALES		
Location and type of tumors	Total Number of Animals with Tumors	Total Mean % Incidence	Range of % Incidence In Studies	Total Number of Animals with Tumors	Total Mean % Incidence	Range of % Incidence In Studies
Brain						
glioma	1	0.1	0- 1.7	0	0	0
Thyroid						
follicular cell adenoma	1	0.1	0- 1.0	0	0	0
adenocarcinoma	0	0	0	1	0.1	0- 1.7
C cell carcinoma	0	0	0	1	0.1	0- 1.7
Parathyroid						
adenoma	0	0	0	2	0.1	0- 3.3
Adrenals						
cortical/medullary adenoma	3	0.2	0- 4.0	7	0.5	0- 1.7
cortical/medullary carcinoma	1	0.1	0- 1.7	4	0.3	0- 3.3
Eyes						
Harderian gland adenoma	39	2.6	0-15.0	10	0.7	0- 3.3
Pituitary						
adenoma	1	0.1	0- 1.7	12	0.8	0- 3.3
Lungs						
adenoma	290	19.5	4.0-35.0	259	17.4	4.0-36.0
carcinoma	95	6.4	0-25.0	107	7.2	0-33.0
rhabdomyosarcoma	0	0	--	2	0.1	0- 1.7
hemangiosarcoma	0	0	--	1	0.1	0- 1.7
sarcoma	1	0.1	0- 1.7	2	0.1	0- 2.0
Stomach						
polyp	3	0.2	0- 3.3	0	0	--
adenocarcinoma	2	0.1	0- 1.7	4	0.3	0- 2.0
squamous cell carcinoma	0	0	--	1	0.1	0- 1.7
hemangiosarcoma	0	0	0	1	0.1	0- 1.7
Salivary glands						
adenocarcinoma	0	0	--	1	0.1	0- 1.7

Location and type of tumors	MALES			FEMALES		
	Total Number of Animals with Tumors	Total Mean % Incidence	Range of % Incidence in Studies	Total Number of Animals with Tumors	Total Mean % Incidence	Range of % Incidence in Studies
Uterus/Cervix (cont.)						
adenocarcinoma				9	0.6	0- 3.3
endometrial stromal sarcoma				3	0.2	0- 1.7
fibrosarcoma				2	0.1	0- 1.7
hemangioma				10	0.7	0- 8.0
hemangiosarcoma				4	0.3	0- 1.7
Vagina						
leiomyoma				1	0.1	0- 2.0
Mammary Gland						
adenoma/fibroadenoma	0	0	--	3	0.2	0- 1.7
adenocarcinoma	1	0.1	0- 1.7	19	1.3	0- 5.0
Skin						
sebaceous gland adenoma	2	0.1	0- 1.7	0	0	--
papilloma	1	0.1	0- 1.7	2	0.1	0- 1.7
squamous cell carcinoma	0	0		1	0.1	0- 1.7
hibernoma	1	0.1	0- 1.0	0	0	--
Subcutis						
hemangioma	4	0.3	0- 3.3	3	0.2	0- 2.0
hemangiosarcoma	3	0.2	0- 1.7	1	0.1	0- 1.7
fibroma	1	0.1	0- 1.7	0	0	--
fibrosarcoma	0	0	--	14	0.9	0- 3.3
Lip						
papilloma	1	0.1	0- 1.0	0	0	--
Pancreas						
islet cell adenoma	1	0.1	0- 1.7	0	0	--
adenocarcinoma	1	0.1	0- 1.7	0	0	--
hemangiosarcoma	0	0	--	1	0.1	0- 1.7
Small Intestine						
adenomatous polyp	0	0	--	1	0.1	0- 1.0
adenocarcinoma	0	0	--	2	0.1	0- 2.0

Location and type of tumors	MALES			FEMALES		
	Total Number of Animals with Tumors	Total Mean % Incidence	Range of % Incidence in Studies	Total Number of Animals with Tumors	Total Mean % Incidence	Range of % Incidence in Studies
Hematopoietic, Lymphatic, Reticuloendothelial Systems						
malignant lymphoma	91	6.1	0-15.0	205	13.8	3.3-27.0
leukemia	9	0.6	0- 5.0	6	0.4	0- 2.0
hemangioma	6	0.4	0- 5.0	4	0.3	0- 3.3
hemangiosarcoma	4	0.3	0- 2.0	4	0.3	0- 3.3
myeloma	2	0.1	0- 1.0	0	0	--
Heart						
fibrosarcoma	0	0	--	1	0.1	0- 1.0
Thymus						
thymoma	1	0.1	0- 1.3	2	0.1	0- 2.0
fibrosarcoma	0	0	--	1	0.1	0- 1.0
Urinary Bladder						
papilloma	1	0.1	0- 1.7	1	0.1	0- 1.0
Bone						
osteoma	0	0	--	1	0.1	0- 1.0
osteosarcoma(skull)	0	0	--	1	0.1	0- 1.7
Seminal Vesicles						
adenoma	2	0.1	0- 1.7			
leiomyosarcoma	1	0.1	0- 1.0			
Skeletal Muscle						
mesothelioma	0	0	--	1	0.1	0- 1.7
Adipose Tissue						
abdominal lipoma	0	0	--	1	0.1	0- 1.0
Vascular Tumors						
hemangioma	5	0.3	0- 3.3	2	0.1	0- 1.7
hemangiosarcoma	2	0.1	0- 1.7	0	0	--

20-22 MONTH STUDIES Location and type of tumors	MALES			FEMALES		
	Total Number of Animals with Tumors	Total Mean % Incidence	Range of % Incidence in Studies	Total Number of Animals with Tumors	Total Mean % Incidence	Range of % Incidence in Studies
Thyroid						
follicular cell adenoma	2	0.4	0- 1.7	1	0.2	0- 1.0
Adrenals						
cortical adenoma	1	0.2	0- 1.7	1	0.2	0- 1.7
cortical carcinoma	0	0		1	0.2	0- 2.0
medullary adenoma	2	0.4	0- 2.0	0	0	--
medullary carcinoma	1	0.2	0- 2.0	0	0	--
Eyes						
Harderian gland adenoma	4	0.8	0- 2.9	0	0	--
Pituitary						
adenoma	0	0	--	3	0.7	0- 3.3
Lungs						
adenoma	89	18.8	4.0-31.1	77	18.3	8.3-28.0
carcinoma	5	1.1	0- 3.3	10	2.4	0- 6.7
Stomach						
adenocarcinoma	1	0.2	0- 1.0	0	0	--
squamous cell carcinoma	0	0	--	2	0.5	0- 2.0
Testes						
hemangioma	3	0.6	0- 3.0			
interstitial cell adenoma	1	0.2	0- 1.7			
epididymis:adenoma	1	0.2	0- 1.0			
Ovaries						
cystadenoma				3	0.7	0- 3.0
papillary adenoma				2	0.5	0- 2.0
teratoma				1	0.2	0- 2.0
Vagina						
polyp				1	0.2	0- 1.7
leiomyoma				2	0.5	0- 1.7

Location and type of tumors	MALES			FEMALES		
	Total Number of Animals with Tumors	Total Mean % Incidence	Range of % Incidence in Studies	Total Number of Animals with Tumors	Total Mean % Incidence	Range of % Incidence in Studies
Spleen						
hemangioma	2	0.4	0- 2.0	0	0	--
hemangiosarcoma	0	0	--	4	1.0	0- 3.0
Liver						
hepatocellular adenoma	3	0.6	0- 4.0	0	0	--
hepatocellular carcinoma	15	3.2	0- 6.0	2	0.5	0- 1.7
hemangioma	4	0.8	0- 3.0	1	0.2	0- 1.7
hemangiosarcoma	3	0.6	0- 4.0	3	0.7	0- 4.0
Kidneys						
adenoma	1	0.2	0- 2.0	0	0	--
Small Intestine						
adenomatous polyp	1	0.2	0- 1.0	0	0	--
adenoma	1	0.2	0- 1.0	0	0	--
Uterus/Cervix						
leiomyoma				6	1.4	0- 3.0
leiomyosarcoma				4	1.0	0- 5.0
polyp				5	1.2	0- 3.0
adenocarcinoma				1	0.2	0- 2.0
hemangioma				2	0.5	0- 2.0
Skin						
papilloma	1	0.2	0- 2.0	2	0.5	0- 2.0
Subcutis						
fibrosarcoma	1	0.2	0- 2.0	0	0	--
Hematopoeitic, Lymphatic, Reticuloendothelial Systems						
malignant lymphoma	30	6.3	2-11.7	40	9.5	3.3-20.0
hemangiosarcoma	1	0.2	0- 1.0	0	0	--
hemangioma	1	0.2	0- 1.0	3	0.7	0- 2.0
myeloma	1	0.2	0- 1.0	2	0.5	0- 4.0
Bone						
osteosarcoma	0	0	--	2	0.5	0- 2.0

Location and type of tumors	MALES			FEMALES		
	Total Number of Animals with Tumors	Total Mean % Incidence	Range of % Incidence in Studies	Total Number of Animals with Tumors	Total Mean % Incidence	Range of % Incidence in Studies
Parathyroid adenoma	0	0	--	1	0.2	0- 2.0
Brain fibrosarcoma	1	0.2	0- 1.0	0	0	--

18 MONTH STUDIES Location and type of tumors	MALES			FEMALES		
	Total Number of Animals with Tumors	Total Mean % Incidence	Range of % Incidence in Studies	Total Number of Animals with Tumors	Total Mean % Incidence	Range of % Incidence in Studies
Adrenals						
cortical adenoma	1	0.3	0- 1.3	0	0	0
Eyes						
Harderian gland adenoma	1	0.3	0- 1.0	1	0.3	0- 1.3
Lungs						
adenoma	41	12.1	6.3-16.7	9	3.0	0- 6.7
carcinoma	5	1.5	0- 6.3	3	1.0	0- 3.8
Testes						
interstitial cell adenoma	1	0.3	0- 1.1			
Spleen						
hemangioma	1	0.3	0- 1.1	0	0	0
hemangiosarcoma	2	0.6	0- 2.5	0	0	0
Liver						
hepatocellular adenoma	8	2.4	0- 8.3	4	1.3	0- 5.0
hepatocellular carcinoma	6	1.8	0- 6.3	1	0.3	0- 1.3
hemangioma	1	0.3	0- 1.7	0	0	0
hemangiosarcoma	1	0.3	0- 1.3	1	0.3	0- 1.3
Uterus						
polyp				6	2.0	0- 3.8
leiomyoma				3	1.0	0- 2.0
leiomyosarcoma				2	0.7	0- 2.5
hemangioma				1	0.3	0- 1.3
hemangiosarcoma				2	0.7	0- 2.5
endometrial stromal sarcoma				1	0.3	0- 1.3
Hematopoietic, Lymphatic, Reticuloendothelial Systems						
malignant lymphoma	14	4.1	1.7-10.0	21	7.0	2.0-13.3
leukemia	1	0.3	0- 1.3	0	0	0
myeloma	3	0.9	0- 2.2	1	0.3	0- 2.0
Subcutis						
fibrosarcoma	1	0.3	0- 1.7	0	0	0

APPENDIX D:

Spontaneous Renal Neoplasms Observed on

18 Food Color Additive Studies

INCIDENCE OF RENAL NEOPLASMS IN CONTROL MALE CD-1 MICE

Study ID[a]	Testing Laboratory	Lesion Description	Incidence	
			Group A	Group B
Blue No. 1	IRD	cortical adenoma	0/60	1/60
Blue No. 2	B/d	tubular cell adenoma	0/57	1/54
Green No. 3	B/d		0/51	0/53
Green No. 5	HL	tubular cell adenoma	1/59	0/59
Yellow No. 5	IRD		0/60	0/60
Yellow No. 6	B/d		0/61	0/60
Yellow No. 10	B/d		0/60	0/60
Orange No. 5	B/d		0/60	0/60
Orange No. 17	B/d	tubular cell adenoma	0/60	2/60
Red No. 3	IRD		0/60	0/60
Red No. 6	IRD		0/60	0/60
Red No. 9	LB	tubular cell adenoma	0/59	2/60
		tubular cell adenocarcinoma	1/59	0/60
		cholesterol granuloma	1/59	0/60
Red No. 19	B/d		0/54	0/57
Red No. 21	IRD	adenoma (N.O.S.)	1/60	0/60
Red No. 27	LB	tubular cell adenoma	1/60	0/59
		hemangiosarcoma	1/60	0/59
Red No. 30	HL		0/60	0/58
Red No. 33	IRD	tubular cell adenoma	1/60	0/60
		cortical carcinoma	1/60	0/60
Red No. 36	LB		0/60	0/60

a - A series of chronic bioassays in Charles River CD-1 mice were conducted on 18 food color additives. These studies were sponsored by the Certified Colors Manufacturers Assoc.; the Cosmetic, Toiletries, and Fragrance Assoc.; and the Pharmaceutical Manufacturers Assoc. Each study utilized 2 concurrent control groups of 60 mice/sex/group. These studies were conducted during the period of 1977 to 1980.

b - Testing laboratories were: International Research and Development Corp. (IRD); Bio/dynamics, Inc. (B/d); Hazleton Laboratories (HL); and Litton Bionetics (LB).

APPENDIX E:

Historical Control Data in CD-1 Mice from

The Institute of Environmental Toxicology

TOXICOLOGIC PATHOLOGY ISSN:0192-6233

Volume 16, Number 3, 1988
Printed in U.S.A.

Mortality, Major Cause of Moribundity, and Spontaneous Tumors in CD-1 Mice*

KEIZO MAITA, MASAHIRO HIRANO, TAKANORI HARADA, KUNITOSHI MITSUMORI, AKIYOSHI YOSHIDA, KIMIMASA TAKAHASHI, NOBUAKI NAKASHIMA, TOSHIAKI KITAZAWA, AKIKO ENOMOTO, KOSEI INUI, AND YASUHIKO SHIRASU

Toxicology Division, Institute of Environmental Toxicology, Suzuki-cho 2-772, Kodaira-shi, Tokyo 187, Japan

ABSTRACT

Mortality, major causes of moribundity, and spontaneous tumors in CD-1 mice were studied in 891 males and 890 females, which were used as controls in 11 different 2-year chronic and oncogenicity studies during the past 5 years. Average mortality of males and females at 83 weeks of age was 32.6% and 28.6%, respectively, and at 109 weeks of age was 66.4% and 63.3%, respectively. Mortality was significantly lowered in males and females born after 1980 in accordance with an abruptly decreased occurrence of systemic amyloidosis in these animals. The major cause of death or moribundity included systemic arteritis, systemic amyloidosis, auricular thrombosis, glomerulosclerosis, lymphoma, and pulmonary adenocarcinoma in both sexes. Dysuria and hepatocellular carcinoma in males and mammary adenocarcinoma in females were also critical lesions. The major tumors occurring at more than 3% incidence were systemic lymphoma, adenoma/adenocarcinoma of the lung, adenoma/carcinoma of the liver and adenoma/adenocarcinoma of the Harderian gland for males, and systemic lymphoma, adenoma/adenocarcinoma of the lung, adenoma/carcinoma of the liver, leiomyoma/leiomyosarcoma of the uterus, adenoma/adenocarcinoma of the pituitary (anterior), adenoma/adenocarcinoma of the mammary gland and adenoma/adenocarcinoma of the Harderian gland for females. Intralaboratory heterogeneities in the incidence were recorded as follows: systemic lymphoma in 1 of 11 control groups (1/11) and adenoma/adenocarcinoma in 1/11 for males, and systemic lymphoma in 3/11, adenoma/adenocarcinoma of the lung in 2/11, adenoma/adenocarcinoma of the liver in 1/11, and adenoma/adenocarcinoma in 1/11 for females.

INTRODUCTION

The mouse is one of the requisite species required for the evaluation of carcinogenic potential of chemicals. B6C3F1 mice have been widely used in this field since they were nominated as the standard strain of the carcinogenicity studies in the middle 1970s by the NCI and NTP programs. A vast amount of historical control data on B6C3F1 mice has been accumulated for mortality and spontaneous tumors during recent years (3, 8, 10). CD-1 mice, also a popular strain in the carcinogenicity studies, have a rather longer history of usage than B6C3F1 mice. They have white hair and white skin which allows easy animal identifications by staining the fur. Ear punch or tag, or toe clipping is less popular because of potential pain to the animals. CD-1 male mice also have much less propensity to fight than male B6C3F1 mice. Owing to these merits, they can be group housed, which saves space and decreases cost of handling. CD-1 mice will continue to be used as one of the major strains of mice in the carcinogenicity studies. Although the histological control data will give a reliable reference in the evaluation of the carcinogenicity studies when an equivocal result is obtained (3), much information has not been available for CD-1 mice (2, 6, 8). While most reports on historical control data have stressed on the incidence of spontaneous tumors, data on mortality and major causes of death or moribundity were generally not available. This type of data is also important for the evaluation of a long term study

The present report includes the mortality and major cause of death or moribundity in untreated control CD-1 mice of both sexes from 11 2-year carcinogenicity studies conducted during the last 5 years. The overall incidence of spontaneous tumors observed is also mentioned in addition to the investigation or variability in the incidence of major tumors.

* Address correspondence to: Dr. Keizo Maita, Toxicology Division, Institute of Environmental Toxicology, Suzuki-cho 2-772, Kodaira-shi, Tokyo 187, Japan.

Methods

Specific pathogen free mice, ICR Crj : CD-1, were purchased from Charles River Japan Inc. (Atsugi, Kanagawa) at 4 weeks of age and transferred to the barrier-sustained animal rooms with a controlled temperature of 24 ± 2°C, humidity 55 ± 10%, lighting of 14 hours light and 10 hours dark, and all fresh air ventilation system circulated at 12 times per hour.

All equipment and materials except for food and water were sterilized before transferring into the animal rooms. After a 1-week acclimatization period, the animals were placed in groups of 3 or 4 of the same sex and housed in a stainless steel cage with a wire mesh floor measuring 215 W × 330 D × 180 H mm. They were allowed free access to powdered chow (Oriental Yeast Co. Ltd., Itabashi, Tokyo) with or without chemical and local tap water through a plastic bottle with glass piece. The standard constitution of nutrients in the powdered chow was as follows: in 100 g of chow, water 7.0 g, crude protein 24.0 g, crude lipid 5.1 g, ash 6.2 g, fiber 3.2 g, soluble non-nitrogen substances 54.5 g, and appropriate amounts of vitamins, generating total calories of 359.9 kcal. Chemical determinations on the drinking water and each batch of the powdered chow were carried out periodically during the study and demonstrated that there were no chemical contaminants, including aflatoxins, pesticides, heavy metals, and others at the concentrations reasonably affecting the conduct of the study. A total of 891 males and 890 females were assigned to the control groups in 11 2-year chronic and carcinogenicity studies delineated as Groups I to XI in the Tables. The initial group size of each control group at the commencement of the study was usually 80 males and 80 females. However, males in Groups III and IV were comprised of 79 and 92, respectively, and females in Groups II, VIII, and XI consisted of 79, 79, and 92, respectively. After 52 weeks of the study, males (8 animals in Group III and 10 in other groups) and females (8 animals in Group III, 9 in Group II or VIII and 10 in other groups) were randomly selected for the interim sacrifice. In Group III, 7 males and 8 females after 26 weeks of the study (31 weeks of age) and 10 males and 10 females after 78 weeks of the study (83 weeks of age) were also sacrificed. The rest of the animals were allowed to live until 104 weeks of the study (109 weeks of age) as the main control group of the chronic and carcinogenicity study.

After necropsy, the following organs and tissues were collected, immersed in neutral buffered 10% formalin, and examined histopathologically: gross lesions including nodules or masses with regional lymph nodes, brain (3 levels), spinal cord (cervical, thoracic, lumbar region), sciatic nerve, pituitary, thymus, thyroids/parathyroids, adrenals, spleen (2 levels), bone with bone marrow (sternum, femur), tibiofemoral joint, lymph node (submandibular, mesenteric), heart (2 levels), thoracic aorta, submandibular gland, tongue, esophagus, stomach (fore- and glandular stomach), duodenum, jejunum, ileum, cecum, colon, rectum, liver (2 levels), pancreas, head (3 levels including nasal cavity, paranasal sinuses, buccal mucosa and middle ear), pharynx, larynx, upper trachea, lung (5 levels including lower trachea), kidneys, urinary bladder, testes, epididymides, prostate, seminal vesicle and coagulating gland, preputial gland, ovaries, oviducts, uterus (cornea, corpus and cervix), vagina, clitoral gland, eyes, Harderian gland, skeletal muscle (M. triceps surae, unilateral), skin (lumbodorsal region), and mammary gland (abdominal region, females only). All tissue sections were stained with hematoxylin and eosin. Other stainings needed for differential diagnoses of the tumors included periodic acid Schiff reaction, azan, alcian blue, toluidine blue, silver-impregnating staining as well as immunohistochemical stainings for desmin, myoglobulin, or S-100 protein using peroxidase anti-peroxidase complex method (PAP). The basic criteria for classification of the tumors were conformed to the text book published by WHO (11).

Heterogeneity of incidence of the lesions among different groups was analyzed by Fisher's exact probability test and the 5% level of probability was used as the criterion of significance.

Results

Mortality and Major Cause of Death or Moribundity

The mortality rates of males or females in each control group are presented in Tables I and II together with the major cause of death or moribundity.

Mortality is shown at 83 weeks and 109 weeks of age corresponding to the period of the termination of the oncogenicity study at 78 weeks and 104 weeks of exposure. Average mortality of males and females at 83 weeks of age was 32.6% and 28.6%, respectively, and at 109 weeks of age was 66.4% and 63.3%, respectively, showing a steep rise during the last half year of the study. There was a tendency for decreasing mortality among groups born after 1930 for both males and females as compared to those born prior to 1980. In almost all control groups, males and females appeared to have a similar rate of mortality. When males showed a considerably higher mortality, females also had a higher rate of unscheduled deaths.

TABLE I.—Mortality and major cause of death or moribundity in male CD-1 mice in control groups from 11 2-year carcinogenicity studies.

	Group											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Total
Birth year and month:	'78, 7	'78, 10	'79, 2	'79, 8	'80, 4	'80, 6	'80, 10	'81, 2	'81, 4	'82, 2	'83, 9	
No. of animals examined[a]:	70	70	64	70	70	70	70	70	70	70	72	766
83 weeks of age	35 (50.0)[b]	32 (45.7)	23 (35.9)	24 (34.3)	16 (22.9)	14 (20.0)	16 (22.9)	15 (21.4)	25 (35.7)	24 (34.3)	26 (36.1)	250 (32.6)
109 weeks of age	56 (80.0)	51 (72.9)	45 (70.3)	50 (71.4)	39 (55.7)	47 (67.1)	39 (55.7)	43 (61.4)	42 (60.0)	48 (68.6)	49 (70.0)	509 (66.4)
Systemic arteritis	0	0	1	2	0	2	1	0	2	1	0	9
Systemic amyloidosis	17	13	3	14	0	0	2	0	0	1	0	50
Auricular thrombosis	13	5	2	4	1	0	2	1	3	4	2	37
Glomerulosclerosis	2	1	2	4	3	2	2	4	3	3	4	30
Dysuria	21	24	21	21	23	10	27	20	21	13	25	226
Lymphoma	8	3	12	4	3	5	4	5	4	5	8	61
Pulmonary adenocarcinoma	13	10	10	9	9	11	3	6	3	14	14	93
Hepatocellular carcinoma	3	3	3	1	2	8	7	6	9	8	1	51

[a] Number of animals designated to main group of each 2-year study excluding the animal served for interim kills from the initial group at commencement of the study.
[b] Percentage mortality shown in parentheses.

TABLE II.—Mortality and major cause of death or moribundity in female CD-1 mice in control groups from 11 2-year carcinogenicity studies.

	Group											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Total
Birth year and month:	'78, 7	'78, 10	'79, 2	'79, 8	'80, 4	'80, 6	'80, 10	'81, 2	'81, 4	'82, 2	'83, 9	
No. of animals examined[a]:	70	70	64	70	70	70	70	70	70	70	72	766
83 weeks of age	24 (34.3)[b]	23 (32.9)	15 (23.4)	28 (40.0)	24 (34.3)	19 (27.1)	12 (17.1)	10 (14.3)	23 (32.9)	28 (40.0)	13 (18.1)	219 (28.6)
109 weeks of age	45 (64.3)	51 (72.9)	44 (62.9)	51 (72.9)	44 (62.9)	46 (65.8)	40 (57.1)	40 (57.1)	42 (60.0)	44 (62.9)	38 (52.8)	485 (63.3)
Systemic arteritis	0	6	3	1	2	4	1	3	7	1	1	29
Systemic amyloidosis	6	16	9	16	4	1	3	0	4	3	0	62
Auricular thrombosis	2	5	2	3	1	1	0	0	1	0	0	15
Glomerulosclerosis	12	4	7	8	14	11	2	9	11	5	9	92
Lymphoma	14	18	19	20	9	16	15	14	6	10	15	156
Pulmonary adenocarcinoma	6	13	11	2	4	5	3	3	6	7	5	62
Mammary adenocarcinoma	4	4	2	6	1	5	3	5	2	6	4	42

[a] Number of animals designated to main group of each 2-year study excluding the animals served for interim kills from the initial group at commencement of the study.
[b] Percentage mortality shown in parentheses.

The major causes of death or moribundity were very similar for males and females including systemic arteritis, systemic amyloidosis, auricular thrombosis, glomerulosclerosis, lymphoma, and pulmonary adenocarcinoma. Dysuria and hepatocellular carcinoma in males and mammary adenocarcinoma in females were also critical lesions in this mouse strain. The diagnosis of systemic arteritis or systemic amyloidosis was given when the lesion was observed in 3 or more different organs or tissues. In the arteritis, the lesions mostly affecting small to medium-sized arteries consisted of marked thickening of the tunica media with a considerable amount of eosinophilic deposits. Thrombosis was also frequently associated with the lesion. The inflammatory reaction was rather mild with a few mononuclear cells, largely lymphocytes, scattered around the affected arteries. The common sites of the lesion were the thymus, ovary, uterus, kidney, and heart.

In the systemic amyloidosis, amyloid showed a predisposition to deposit in the stroma of the glandular tissue, lymphoid tissue, or genital organs. Thyroid, adrenal, kidney, small intestine, and ovary were the predilection sites of the deposition. It was rather rare to see systemic amyloidosis and systemic arteritis in the same animal. The occurrence of the systemic amyloidosis was remarkably changed between Groups IV and V in both sexes (Table II). The systemic amyloidosis was one of the most common diseases among Groups I to IV in both sexes except males in Group III. However, in the groups born after April 1980, the lesion became a rare disease. The decreased incidence was more drastic in males.

Auricular thrombosis largely seen in the left auricle was rarely associated with systemic arteritis and other vascular changes. The lesion appeared to occur more frequently in males than females, and the incidences seemed to be lowered among the animals born after April 1980.

Glomerulosclerosis of the kidney included a considerably wide range of glomerular changes along with a variety of regressive alterations of the proximal tubules and inflammatory reactions in stroma. In the glomeruli, there were abundant eosinophilic deposits which were largely hyaline material but sometimes were amyloid-like substances. Sclerotic changes were not usually conspicuous. With increasing the severity of the lesion, regressive changes of the proximal tubules including vacuolation, atrophy, and necrosis of the epithelial cells became prominent in association with hyaline cast deposits in the lumen. There was a small amount of infiltration of lymphocytes or macrophages and a slight proliferation of the connective tissue in stroma. In advanced cases, dilatation of the pelvis was a ubiquitous finding in the lesion, and at this stage the disease was listed as a probable cause of death or moribundity of the animal. Females were 3 times more vulnerable to the disease than males.

Dysuria or urinary retention was the most destructive and fatal disease in males and nearly 30% of males died of this disease during the study. On laparotomy, the urinary bladder was distended with urine often more than 1 cm in diameter together with marked hypertrophied seminal vesicles and coagulating glands. The urethra was almost completely obstructed just before the isthmus urethrae by whitish, gelatinous, plug-like material in which numerous sperm were easily identified histologically. The affected animals showed lethargy, ruffled hair, anemia, shivering, and marked physconia with blotted fur on the lower abdomen. These symptoms appeared suddenly without any history of physical disorder and usually lasted for only 1 night or 1 day before death. There were no significant histological changes in the pituitary or testis and the central or peripheral nervous system, although the urethral embolism with plug-like material was observed along with marked retention of secrete in the seminal vesicle and coagulating gland. The incidence of this disease was almost comparable among the different controls except for Groups VI and X in which the incidence was lower.

Lymphoma was the most significant lesion among the probable causes of death or moribundity in females. On the average, 20% of females were emaciated during the study by lymphoma while only 8% of males were affected. Pulmonary adenocarcinoma was also one of the malignant tumors commonly seen in both males and females with a slightly predominant occurrence in females. Although a slightly higher incidence was noted for hepatocellular carcinoma in Groups VI to X, there was no variation in the incidences of major malignant tumors among the different control groups of both sexes found dead or killed in extremis during the studies.

Spontaneous Tumors

The types and incidences of spontaneous tumors observed in the control groups of both sexes from 11 different carcinogenicity studies for a period of 2 years were shown in Table III.

Cardiovascular System. Only 1 tumor observed in the cardiovascular system among a total of 1,781 CD-1 mice was mesothelioma in the heart of a male.

Hematopoietic System. Lymphoma was the most prominent tumor in the hematopoietic system. Females were more predisposed to the tumor. The incidences in females were twice greater than those in males for thymic or splenic lymphoma, and for systemic lymphoma the predominance of females

TABLE III.—Incidences of spontaneous tumors in male and female CD-1 mice in control groups from 11 2-year carcinogenicity studies.

No. of animals examined[a]	Male 891	Female 890
Cardiovascular system		
Heart:		
Mesothelioma	1	0
Hematopoietic system		
Thymus:		
Thymoma	1	0
Lymphoma	5	11
Spleen:		
Hemangioma	5	7
Hemangiosarcoma	4	6
Lymphoma	4	8
Malignant histiocytoma	1	1
Osteoma	0	1
Lymph node:		
Hemangioma	1	0
Hemangiosarcoma	0	1
Lymphoma	5	5
Systemic:		
Lymphoma	72	196
Myeloid leukemia	16	17
Malignant mastocytoma	2	0
Leukemia (not classified due to autolysis)	1	1
Respiratory system		
Nasal cavity:		
Squamous cell carcinoma	0	1
Lung:		
Adenoma	130	129
Adenocarcinoma	168	108
Hemangioma	0	1
Digestive system		
Oral cavity:		
Papilloma	1	0
Salivary gland:		
Adenoma in parotid gland	0	2
Adenoma in submaxillary gland	2	0
Stomach:		
Adenoma in glandular stomach	1	0
Squamous cell carcinoma in forestomach	3	1
Malignant histiocytoma	0	1
Small intestine:		
Adenoma	2	1
Adenocarcinoma	0	2
Hemangioma	0	1
Lymphoma	0	2
Sarcoma (unclassified)	1	0
Large intestine:		
Squamous cell carcinoma	0	1
Sarcoma (unclassified)	1	0

TABLE III.—Continued

No. of animals examined[a]	Male 891	Female 890
Anus:		
Adenoma	1	0
Squamous cell carcinoma	1	0
Liver:		
Hepatocellular adenoma	235	46
Hepatocellular carcinoma	81	8
Hepatoblastoma	4	0
Cholangioma	0	1
Cholangiocarcinoma	0	1
Hemangioma	13	4
Hemangiosarcoma	4	1
Malignant histiocytoma	1	3
Gallbladder:		
Papilloma	1	1
Pancreas:		
Acinar cell adenoma	1	0
Islet cell adenoma	3	5
Fibrosarcoma	1	0
Urinary system		
Kidney:		
Adenoma	3	1
Adenocarcinoma	1	1
Carcinoma	2	0
Lipoma	1	0
Lymphoma	1	0
Urinary bladder:		
Transitional cell carcinoma	1	0
Genital system		
Testis:		
Interstitial cell tumor	8	—
Papillary adenoma	1	—
Stromal tumor	1	—
Epididymis:		
Hemangioma	1	—
Malignant fibrous histiocytoma?	1	—
Seminal vesicle:		
Adenoma	1	—
Adenocarcinoma	1	—
Coagulation gland:		
Adenoma	3	—
Prostate:		
Adenocarcinoma	1	—
Preputial gland:		
Adenoma	1	—
Ovary:		
Papilloma	—	1
Adenoma	—	10
Theca cell tumor	—	4
Luteoma	—	6
Granulosa cell tumor	—	2
Malignant granulosa cell tumor	—	1
Hemangioma	—	4
Hemangiosarcoma	—	1

TABLE III.—Continued

No. of animals examined[a]	Male 891	Female 890
Oviduct:		
Papilloma	—	1
Leiomyoma	—	1
Uterus:		
Papilloma	—	5
Adenoma	—	5
Adenocarcinoma	—	2
Fibroma	—	1
Endometrial sarcoma	—	2
Hemangioma	—	8
Hemangiosarcoma	—	1
Leiomyoma	—	21
Leiomyosarcoma	—	12
Malignant histiocytoma	—	1
Vagina:		
Leiomyosarcoma	—	1
Endocrine system		
Pituitary:		
Anterior adenoma	2	42
Anterior adenocarcinoma	2	2
Thyroid:		
Adenoma	8	6
Parathyroid:		
Adenoma	0	1
Adrenal:		
Cortical adenoma	8	4
Cortical adenocarcinoma	0	3
Pheochromocytoma	5	3
Malignant pheochromocytoma	0	1
Undifferentiated sarcoma	1	3
Skin, subcutis, mammary gland, Harderian gland		
Skin, subcutus:		
Papilloma	2	0
Keratoacanthoma	1	1
Squamous cell carcinoma	2	6
Basal cell carcinoma	0	1
Fibroma	1	3
Fibrosarcoma	15	8
Liposarcoma	0	7
Hemangioma	4	8
Hemangiosarcoma/malignant hemangiopericytoma	3	7
Leiomyosarcoma	0	1
Rhabdomyosarcoma	4	1
Malignant mastocytoma	0	1
Malignant histiocytoma	0	1
Malignant fibrous histiocytoma?	3	4
Sarcoma (unclassified)	0	1
Mammary gland:		
Adenoma	1	9
Adenocarcinoma	0	56
Harderian gland:		
Adenoma	91	61
Adenocarcinoma	4	2

TABLE III.—Continued

No. of animals examined[a]	Male 891	Female 890
Auricle:		
Fibrosarcoma	0	1
Musculoskeletal system		
Muscle:		
Hemangioma	1	0
Hemangiosarcoma	1	0
Bone:		
Osteoma	0	2
Osteosarcoma	2	2
Body cavity		
Cranial cavity:		
Osteoma	1	0
Malignant meningioma	1	0
Malignant schwannoma	1	0
Thoracic cavity:		
Malignant histiocytoma	1	0
Abdominal cavity:		
Malignant mesothelioma	0	1
Hemangioma	0	3
Hemangiosarcoma	1	1
Lipoma	1	1
Liposarcoma	0	1
Rhabdomyosarcoma	1	0
Osteosarcoma	0	1

[a] Total number of animals at commencement of each study including the animals served for main group of 2-year study and subjected to interim kills during the study.

[b] Percentage to total number of animals examined is shown in parentheses.

was 2.7 times over the males. The thymic lymphoma was exclusively the lymphocytic type and was seen in younger animals. The splenic lymphoma was also the lymphocytic type, but occurred rather in senescence. Systemic lymphoma was given as the diagnosis when at least 3 different organs/tissues or 3 different locations of the lymph node were affected by the neoplastic cells. Systemic lymphoma included 3 types: lymphocytic, histiocytic, and composite. The systemic lymphocytic lymphoma occurred from young age. Although the primary target site of this type of lymphoma was the lymphatic tissues, the neoplastic cells showed a prominent proliferation in the abdominal, pleural cavity, and/or subcutis without any intensive invasion to the lymphatic tissues in some cases. These cases were only observed in old animals. The histiocytic and composite lymphomas were also the disease in senescence. The predilection site of the tumor cells was limited in the organs/tissues in the abdominal and pleural cavity, but was rarely extended to the lymphatic tissues of the skin.

Myeloid leukemia occurred largely during the first year with a similar incidence in both males and females.

Respiratory System. Adenoma and adenocarcinoma of the lung were the most frequent tumors in females and second only to hepatocellular adenoma/carcinoma in males. While the proliferating cells of the tumors found in younger animals mostly resembled the alveolar cells with faintly eosinophilic, foamy cytoplasm and oval nuclei, tumor cells in older animals tended to accentuate characteristics of the bronchiolar cells which were smaller in size and had basophilic cytoplasm and chromatin-rich nuclei. Adenocarcinoma exclusively consisted of the latter cells and occasionally metastasized to the mediastinum, thoracic wall, diaphragm, heart, kidney, and other organs in the abdominal cavity. It was rather frequent that plural tumor nodules with different histological features originated separately in the lung.

Digestive System. For hepatocellular adenoma and carcinoma, males were more predisposed to the tumor than females and the incidence in males, 35.5%, was nearly 6 times greater than that in females, 6.1%. Among 81 cases of hepatocellular carcinoma in males, 7 had metastasized to the lung.

The occurrence of neoplastic lesions in the digestive system other than the liver was extremely rare in both males and females.

Urinary System. Neoplastic lesions in the urinary system were very rare in CD-1 mice. The incidence of the epithelial tumors in the kidney was 6/891 (0.7%) in males and 2/890 (0.2%) in females, respectively.

Genital System. The interstitial cell tumors occurred in 8 of 891 males (0.9%) and were seen grossly as small nodules which rarely occupied more than half of the testis. The tumor cells were spindle shaped containing darkly eosinophilic and granular or vesicular cytoplasm and oval to elongated nuclei.

In the ovary, adenomas which probably originated from the surface (germinal) epithelium, or the glandular tissue at the hilus were most frequently seen.

In old females, the endometrium of the uterus was sometimes greatly activated and the epithelial cells showed a papillary or cystic proliferation into the lumen together with hyperplasia of the glandular components. These hyperplastic lesions of the endometrium often contained the proliferating foci of the smooth muscle. In advanced cases where the proliferation of the smooth muscle cells become predominant in the lesions, leiomyoma or leiomyosarcoma was given as the diagnosis depending on the degree of atypia of the cytoplasm and/or nucleus of the tumor cells.

Skin/Subcutis. As with soft tissue tumors arising from other parts of the body, the diagnosis of the subcutaneous tumors of mice was sometimes more troublesome because the tumor cells, especially those with malignant features in histology, tended to change into spindle shaped or fibroblastic type cells or to show higher pleomorphism without histological characteristics suggestive of their original cell type. In the present study, oil red O staining was used to differentiate liposarcoma, and immunohistochemical staining for desmin and myoglobin was applied to identify myogenic sarcomas. S-100 protein was used for the tumors of peripheral nerve origin. Although the tumor cells of rhabdomyosarcoma in this series revealed neither cross striations by phosphotungstic acid staining nor Z-band structure in electron microscopy, a PAP procedure with antibody against rat myoglobin could successfully demonstrate the positive fine particles in the tumor cells. The term of malignant fibrous histiocytoma was tentatively given to the tumors with the following features: a high pleomorphism of the tumor cells with frequent formation of giant cells, presence of gigantic or bizarre nuclei, no positive reaction to immunohistochemical staining for desmin, myoglobin, or S-100 protein, and considerably abundant proliferation of collagen fibers sometimes occupying a large area of a tumor mass.

Mammary Gland. Among 65 cases of mammary gland tumors in females, 59 cases were adenocarcinoma, and 14 of them (23.7%) showed metastasis to the remote organs, mainly to the lung. Intraductal papillary proliferation was the most dominant pattern in the adenocarcinomas.

Harderian Gland. Harderian gland tumors occurred frequently in both males and females at incidences of 10.7% and 7.1%, respectively. The tumors sometimes arose bilaterally. Although most of the tumors were benign, a total of 6 cases were diagnosed as adenocarcinoma in both sexes and 1 papillary adenocarcinoma in a male of 106 weeks of age metastasized to the lung.

Variability in the Incidence of Major Spontaneous Tumors

The incidences of major spontaneous tumors showing more than 3% incidence among 11 control groups were shown in Table IV for males and Table V for females. In males, significant heterogeneities were noted in lower incidences of systemic lymphoma in Group V and adenoma/adenocarcinoma of the Harderian gland in Group VII. In females, lower incidences were seen in lymphoma of Group V or IX, and in adenoma/adenocarcinoma of the lung in Group IV, and in adenoma/adenocarcinoma of the Harderian gland in Group IX. Significant het-

erogeneities in females were also demonstrated in higher occurrences of systemic lymphoma in Group IV, adenoma/adenocarcinoma of the lung in Group V, and adenoma/carcinoma of the liver in Group III.

Discussion

One of the most interesting changes found in the present investigation was the difference in mortality seen in CD-1 mice of both sexes born before 1979 as compared with those born after 1980. It is clear that the decreased mortality in recent years was attributable to the lower occurrence of systemic amyloidosis. There is a common recognition among pathologists that ICR mice are predisposed to amyloidosis (2). Homburger et al (5) reported that amyloid deposition was observed in 44 of 56 males and 43 of 54 females in CD-1 HaM/ICR mice surviving up to 18 months of age. The occurrence of spontaneous lesions was influenced by various factors including genetic background, dietary factors, housing conditions, husbandry procedures, infectious disease and so on. These factors have been well analyzed on the tumor incidence and reviewed by several authors (1, 4). While the incidence of a certain type of tumor is sometimes abruptly changed in mice, even in an inbred strain, Hoag (4) mentioned an interesting example of this case with DBA/2J mice which showed an abrupt decrease in the incidence of mammary adenocarcinoma from 50% to nearly 0% after an episode of a breeding slump lasting for a period of 2 years, suggesting that some significant changes occurred in the genetic background of this strain of mice. In our laboratory, only Charles River CD-1 mice showed a significant decrease in the occurrence of amyloidosis after 1980, while other ICR mice from different sources still have a high rate of this disease. Since the housing condition, handling procedures, diet, etc. are all the same in our laboratory for ICR mice of both Charles River and other origins, it is plausible that some changes beneficial to prevention of amyloidosis happened to occur in the genetic background of the breeding colony of CD-1 mice. Another interesting finding is that Group III males born in February 1979 showed relatively lower incidences of amyloidosis than Group IV born in August 1979 suggesting that there were 2 separate lines of the breeding colony coexisting in the breeder and that the 1 line responsible for amyloidosis was discarded in late 1979. This change may have no influence on the occurrence of other diseases including spontaneous tumors, since there were no significant changes in the incidence of these lesions as indicated in Tables I, II, IV, and V.

The types of the spontaneous tumors were the

Table IV.—Variability of major spontaneous tumors at more than 3% incidence among 11 control groups in male CD-1 mice.

	Group											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Total
Birth year and month:	'78, 7	'78, 10	'79, 2	'79, 8	'80, 4	'80, 6	'80, 10	'81, 2	'81, 4	'82, 2	'83, 9	
No. of animals examined[a]:	80	80	79	80	80	80	80	80	80	80	92	891
Systemic lymphoma	7	6	12	5	2[b]	7	6	8	5	5	9	72
Lung:												
Adenoma + adenocarcinoma	23	29	26	21	28	26	17	35	26	27	40	298
Liver:												
Adenoma + carcinoma	25	25	21	32	27	24	27	33	28	33	41	316
Harderian gland:												
Adenoma + adenocarcinoma	11	12	10	4	7	13	3[b]	8	8	7	12	95

[a] Total number of animals at commencement of each study including the animals served for main group of 2-year study and subjected to interim kills during the study.
[b] Significant heterogeneity $p < 0.05$.

TABLE V.—Variability of major spontaneous tumors at more than 3% incidence among 11 control groups in female CD-1 mice.

	Group											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Total
Birth year and month:	'78, 7	'78, 10	'79, 2	'79, 8	'80, 4	'80, 6	'80, 10	'81, 2	'81, 4	'82, 2	'83, 9	
No. of animals examined[a]:	80	79	80	80	80	80	80	79	80	80	92	890
Systemic lymphoma	18	21	16	27[b]	9[b]	18	22	21	8[b]	11	25	196
Lung:												
Adenoma + adenocarcinoma	16	20	26	14[b]	31[b]	18	22	27	19	19	25	237
Liver:												
Adenoma + carcinoma	6	2	10[b]	6	3	3	5	7	2	7	3	54
Uterus:												
Leiomyoma + leiomyosarcoma	4	3	4	3	1	0	6	2	5	2	3	33
Pituitary (anterior):												
Adenoma + adenocarcinoma	5	3	6	2	2	3	5	5	2	8	3	44
Mammary gland:												
Adenoma + adenocarcinoma	6	4	4	9	3	5	5	7	7	10	5	65
Harderian gland:												
Adenoma + adenocarcinoma	9	4	8	5	4	8	2	8	1[b]	6	8	63

[a] Total number of animals at commencement of each study including the animals served for main group of 2-year study and subjected to interim kills during the study.
[b] Significant heterogeneity, $p < 0.05$

same as those reported in CD-1 (2, 5, 6, 8) or other strains of mice (4, 7, 9, 11). The incidences of lymphoma, 8.8% in males and 22.0% in females, were comparable to those of higher incidence groups in CD-1 mice reported by Sher et al (8) and were lower than those by Homburger et al (5) and Percy et al (6). The CD-1 mice in the present study seemed to belong to the relatively higher incidence group as to lymphoma (4, 8, 9). Although Percy reported extremely lower incidences (3.0–7.5%) of pulmonary tumors in 4 of 5 groups studied (6), the present results, 33.4% in males and 26.6% in females, were almost comparable to those of other reports on CD-1 mice (5, 8), and nearly the same as those of higher incidence groups of lung tumors among various mouse strains (4). One difference in this study as compared to previous reports on CD-1 mice was the higher incidence of liver adenoma and adenocarcinoma in males. In the previous reports, the highest incidence of hepatocellular adenoma and adenocarcinoma in male CD-1 mice was 20% (a combined incidence) (8). However, the combined incidence in the present study was greater than 35% and nearly comparable to that of male B6C3F1 mice which are well known to have a high incidence of hepatocellular tumors (8, 10). On the other hand, the incidence of the hepatocellular tumors in female mice in the present study was within the range of variation reported by Sher et al (8). The incidences of other tumors in males and females were almost comparable to those reported by Homburger et al (5) except on a slightly higher occurrence of pituitary tumors in females.

The occurrence of spontaneous tumors is influenced by various factors including genetic background, food, housing, infection, hormone, age, and so on as reviewed by Everett (1). In addition to these factors, the tumor incidences are greatly fluctuated by diagnostic criteria of each pathologist. Recently, much concern has focused on the intralaboratory variability of spontaneous tumor incidences and increasing questions have been raised concerning the feasibility of using historical control data in the evaluation of carcinogenicity studies (3, 10). In the present study, the total incidences of adenoma and adenocarcinoma arising from major glandular tissues including lung, liver, pituitary, mammary gland, and Harderian gland were strikingly comparable among 11 different control groups of both sexes. As for systemic lymphoma which is not a diagnostic problem, the incidences from 11 different control groups showed significant heterogeneities only rarely. There was no significant heterogeneity in the uterine myogenic tumors. The present result might indicate that although the occurrence of spontaneous tumors was greatly influenced by various factors, the data from control groups accumulated during recent years would be useful as the historical control data in the laboratory.

Acknowledgments

We wish to express our acknowledgment to Dr. G. A. Boormann, Chief of Chemical Pathology Branch, NIEHS, Research Triangle Park, North Carolina, for revision of this manuscript with useful suggestions. We also thank Mrs. S. Goda for her patience and persistence in typing this manuscript.

References

1. Everett R (1984). Factors affecting spontaneous tumor incidence rates in mice. A literature review. *CRC Crit. Rev.* 13: 235–251.
2. Glaister JR (1986). The ageing mouse. In: *Principles of Toxicological Pathology.* Taylor & Francis, London and Philadelphia, pp. 182–203.
3. Haseman JK, Huff J, and Boorman GA (1984). Use of historical control data in carcinogenicity studies in rodents. *Toxicol. Pathol.* 12: 125–135.
4. Hoag WG (1963). Spontaneous cancer in mice. *Ann. N.Y. Acad. Sci.* 108: 805–831.
5. Homburger A, Russfield AB, Weisburger JH, et al (1975). Aging changes in CD-1 HaM/ICR mice reared under standard laboratory conditions. *J. Natl. Cancer Inst.* 55: 37–45.
6. Percy DH and Jonas AM (1971). Incidence of spontaneous tumors in CD-1 HaM/ICR mice. *J. Natl. Cancer Inst.* 46: 1045–1065.
7. Sheldon WG and Greenman DL (1979). Spontaneous lesions in control BALB/C female mice. *J. Environ. Pathol. Toxicol.* 3: 155–167.
8. Sher SP, Jensen RD, and Bokelman DL (1982). Spontaneous tumors in control F-344 and Charles River-CD rats and Charles River CD-1 and B6C3F1 mice. *Toxicol. Lett.* 11: 103–110.
9. Smith GS, Walford RL, and Mickey MR (1973). Lifespan and incidence of cancer and other diseases in selected long-lived inbred mice and their F1 hybrids. *J. Natl. Cancer Inst.* 50: 1195–1213.
10. Tarone RE, Chu KC, and Ward JM (1981). Variability in the rates of some common naturally occurring tumors in Fischer 344 rats and (C57BL/6N × C3H/HeN)F1 (B6C3F1) mice. *J. Natl. Cancer Inst.* 66: 1175–1181.
11. Turusov VS, Porta GD, Hollander CF, et al (1979). *Pathology of Tumors in Laboratory Animals,* Vol. II, *Tumors of the Mouse.* International Agency for Research on Cancer, Lyon.

13544

037348

Chemical:	**Glyphosate**
PC Code:	**417300**
HED File Code	**13000 Tox Reviews**
Memo Date:	**06/19/89**
File ID:	**TX007252**
Accession Number:	**412-03-0108**

HED Records Reference Center
04/02/2003