Glyphosate / Tox

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

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MEMORANDUM 2-15-83

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Robert Taylor (25)
Registration Division (TS-767)

THRU: Orville E. Paynter, Ph.D. OEP 2/10/83
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769)

SUBJECT: Glyphosate; EPA Reg.#524-308; A Lifetime Feeding Study of Glyphosate in Sprague-Dawley Rats; A Preliminary Addendum to Review dated 2/18/83 CASWELL#661A

Dr. Len Ritter of the Canadian HPB telephoned to request Toxicology Branch's interpretation of the significance of the incidence of C-cell carcinomas of the thyroid in female rats in the lifetime feeding study in rats with Glyphosate. No reference to these tumors was made in the review of 2/18/83.

Table I presents the incidence (percent) of females bearing C-cell tumors at terminal sacrifice and Table II presents the incidence (percent) of females bearing thyroid C-cell tumors of all animals examined.

TABLE I: GLYPHOSATE: LIFETIME FEEDING STUDY IN SPRAGUE-DAWLEY RATS
(BDN-77-416; B/d No. 77-2062)

INCIDENCE (PERCENT) OF SPRAGUE-DAWLEY FEMALES BEARING THYROID C-CELL TUMORS AT TERMINAL SACRIFICE

GROUP	CONTROL	LOW-DOSE	MID-DOSE	HIGH-DOSE
TUMOR	0	3(mg/kg/day)	10	30
ADENOMA	2/18 (11)*	2/23 (9)	4/30 (13)	1/14 (7)
CARCINOMA	1/18 (6)	0/23 (0)	1/30 (3)	2/14 (14)
ADENOMA or CARCINOMA	3/18 (17)	2/23 (9)	5/30 (17)	3/14 (21)

*Percentage is in parenthesis.

TABLE II: GLYPHOSATE: LIFETIME FEEDING STUDY IN RATS
(BDN-77-416: B/d No. 77-2062)

INCIDENCE (PERCENT) OF SPRAGUE-DAWLEY FEMALES BEARING THYROID C-CELL TUMORS OF ALL ANIMALS EXAMINED

GROUP	CONTROL	LOW-DOSE	MID-DOSE	HIGH-DOSE
TUMOR	0	3(mg/kg/day)	10	30
ADENOMA	5/47 (11)	3/49 (6)	6/50 (12)	3/47 (6)
CARCINOMA	1/47 (2)	0/49 (0)	2/50 (4)	6/47 (13)
ADENOMA or CARCINOMA	6/47 (13)	3/49 (6)	8/50 (16)	9/47 (19)

Table I shows that the percent incidence of carcinomas is higher in the high-dose females (14%) than in the control females (6%) at terminal sacrifice. The percent incidence of adenoma or carcinoma presented in Table I shows a comparable incidence between control (17%) and high-dose females (21%) at terminal sacrifice.

Table II shows that the percent incidence of carcinomas for all female animals examined is 2% in the controls and 13% in the high-dose animals. Additionally, the percent incidence of adenoma or carcinoma in Table II shows that the controls (13%) are comparable to the high-dose (19%).

The time to tumors data also shows that the latency of tumors is not affected by treatment (Table III). Thyroid weights showed no treatment-related increases and thyroid tumors were not grossly observed except for female rat #831 which had thyroid carcinoma.

TABLE III: TIME TO TUMOR DATA OF ANIMALS/MORIBUND SACRIFICE AND DIED ON STUDY/SPRAGUE-DAWLEY FEMALE THYROID TUMORS

Group I - Controls

Animal Number	Tumors	Days	Weeks
225	Adenoma	702	100.3
229	Adenoma	629	89.9
234	Adenoma	699	100.0

Group II - Low-Dose

Animal Number	Tumor	Days	Weeks
443	Adenoma	703	100.4

Group III - Mid- Dose

Animal Number	Tumor	Days	Weeks
618	Adenoma	748	106.9
638	Adenoma	605	86.4
641	Carcinoma	677	96.7

Group IV - High-Dose

Animal Number	Tumor	Days	Weeks
803	Adenoma	689	98.4
820	Carcinoma	751	107.3
822	Adenoma	751	107.3
831	Carcinoma	778	111.1
834	Carcinoma	734	104.9
835	Carcinoma	652	93.1

Table IV presents the Bio/dynamics thyroid C-cell tumor historical control data on female Charles River albino (CD) rats.

TABLE IV: BIO/DYNAMICS THYROID C-CELL TUMOR HISTORICAL CONTROL DATA: FEMALE CHARLES RIVER ALBINO (CD) (SPRAGUE-DAWLEY RATS)

INCIDENCE (PERCENT) OF FEMALES BEARING THYROID C-CELL TUMORS ALL ANIMALS SACRIFICED POST 12-MONTHS

STUDY	ADENOMA or CARCINOMA	ADENOMA	CARCINOMA
B			
Group A*	10/58 (17)	10/58 (17)	0/58 (0)
Group B	7/59 (12)	6/59 (10)	1/59 (2)
C			
Group A	5/59 (8)	5/59 (8)	0/59 (0)
Group B	6/85 (10)	6/58 (10)	0/58 (0)
I			
Group A	9/57 (16)	6/57 (11)	3/57 (5)
Group B	6/55 (11)	5/55 (9)	1/55 (2)
J			
Group A	2/58 (3)	2/58 (3)	0/58 (0)
Group B	0/55 (0)	0/55 (0)	0/55 (0)
L	1/53 (2)	1/53 (2)	0/53 (0)

*Studies #B, C, I and J had two control groups per study, identified as Group A or B.

The historical control data from Bio/dynamics presented in Table IV shows that the percent incidence of carcinomas varied from 0-5%, whereas the percent incidence of adenomas or carcinomas varied from 0-17%.

Literature sources on C-cell thyroid tumors has been researched and provide the following information in Tables V through X.

A spontaneous incidence of 22% C-cell tumors in Sprague-Dawley rats has been reported as shown in Table V (Table 12.2, Page 1056).

Table V and VI present the incidence of C-cell tumors in various strains of rats from published literature.

TABLE V: TUMORS OF RAT STRAINS THYROID, PARAFOLLICULAR CELL*

Strain	Average Incidence (%)	(Months)	Comments
Buffalo	25	> 24	increase with age
Fischer	22	> 24	increase with age
Long-Evans	12-45	> 24	increase with age
OM	33	> 24	increase with age
Sprague-Dawley	22	> 24	increase with age
Wistar	19	> 24	increase with age

*Benvischke et al, Reference 1

Also, Tables V and VI shows the spontaneous incidence of C-cell tumors in other strains of rats.

TABLE VI: PATHOLOGY OF AGING RATS*

Summary of the Incidence of Medullary Thyroid Carcinomas and Metastases of Medullary Thyroid Carcinomas in Aging BN/Bi, WAG/Rig, and (WAG x BN) F_1 Rats,*

Strain	Sex	Number Examined	No. with medullary thyroid carcinoma	Percent	Mean age (range) in months	No. medullary thyroid carcinoma with metastases	Age (in months) of rats with metastatic medullary thyroid carcinomas
Bn/Bi	Female	236	15	6	33 (17-38)	2	35, 38
	Male	74	7	9	27 (15-34)	0	-
WAG/Rij	Female	101	47	47	35 (26-46)	5	35 (32-39)
	Male	124	41	33	23 (9-29)	1	29
F_1	Female	68	11	16	31 (17-38)	3	25, 27, 28
	Male	67	20	29	34 (22-42)	3	28, 30, 38

*Burek (1978), Reference 2

These references show a high spontaneous incidence of C-cell tumors in various strain of rats.

More specific literature sources revealed the following information:

Thompson and Hunt (1963) showed the following results:

TABLE VII

SUMMARY OF SPONTANEOUS TUMORS OBSERVED UPON RE-EXAMINATION OF SERIAL SECTIONS OF SELECTED TISSUES FROM 117 (63 MALES, 114 FEMALES) SPRAGUE-DAWLEY RATS

Type of tissue and tumor	No. of organs examined	Number of Tumors				Age in Days
		Single Section		Serial Section		
		Male	Female	Male	Female	
Thyroid light cell adenoma	140	4	5	24	31	300-960

The following quote is taken from their 1963 publication and illustrates the increase in tumors found by serial sectioning. "As depicted in Table 1, (Table VII, above) a total of 55 lightcell adenomas (24 males, 31 females) were encountered upon re-examination of serial tissue sections of 140 thyroid glands (54 males, 86 females). Only nine of these tumors (four males, five females) were originally observed in single random tissue sections of the thyroid glands of 177 rats (63 males, 114 females). All the nodules were of similar histologic structure, being composed of epithelial cells with leptochromatic nuclei, surrounded by a pale, slightly eosinophilic cytoplasm. Mitotic figures were not common, and

the cells tended to be organized into lobules. Follicles were not formed by the tumor cells. However, small colloid filled follicles were frequently seen within the substance of these tumors, but were thought to represent normal thyroid follicles which had become encompassed as the tumors enlarged. These nodules varied in size from a microscopic collection of light-cells to large nodules which almost completely replaced the thyroid gland. The smaller nodules were always observed in the central portion of the gland; never occurring at the periphery or in the isthmus. The nodules were frequently encountered in both lobes of the thyroid. The age range of the rats in which light-cell adenomas were observed was 300 to 960 days with a mean of 637 days."

Mackenzie and Garner (1973) presented the following information which shows the difficulty in assessing endocrine adenomas and carcinomas.

"A neoplasm was defined as a lesion with cellular architectural change; it expanded and compressed surrounding tissue noticeably. Size of tumor was not a criterion, if compressed tissue was demonstrable. Many tumors were microscopic and found on a single random section of each organ. No attempt was made to cut deeper into the blocks available, on the chance that additional small neoplasms might be uncovered. The criterion used to diagnose malignancy was the evidence of growth by invasion and/or metastasis. As the material submitted was often inadequate to demonstrate invasion, those tumors morphologically similar to known malignant tumors of the same type were also considered malignant. Neoplasms of the endocrine system, however, could not be classed accurately as benign or malignant by histology, and these are simply called adenomas."

MacKenzie and Garner (1973) examined six sources of rats and found the following results:

TABLE VIII: Sources of Rats

Source and Identification	Number of Rats	Remarks
Sprague-Dawley Inc. (Sprague-Dawley).	258	Colony originated in 1929. Closed colonies, random breeding.
Charles River Inc. (Charles River-SD).	535	Original stock from Sprague-Dawley Inc. Selectively randombred.
Holtzman Inc. (Holtzman-SD).	208	Nucleus stock from Sprague-Dawley in 1946. Closed colony selectively randombred.
Diablo Animal Laboratories (Diablo-SD).	217	Nucleus stock from Holtzman Inc. (Sprague-Dawley strain). Maintained closed colony, selectively randombred.
Locally bred (Osborne-Mendel).	131	Nucleus stock from Food and Drug Administration Washington, D.C. Bred as closed colony for 2 years for project.
Locally bred (Oregon).	673	Closed colony for over 30 years. Random breeding. Original stock of unknown origin.

TABLE IX: Tumors and Organs of Origin in 2,082 Rats of 6 Sources*

Tumors	Sprague-Dawley	Holtz-man-SD	Charles River-SD	Diablo-SD	Osborne-Mendel	Oregon	Total
Number of Rats	258	268	535	217	131	673	2,082
Thyroid:							
Light-cell adenoma	15	9	12	8	2	3	49
Follicular cell carcinoma			2				2

*MacKenzie and Garner (1973), Reference 7

Suzuki et al (1979) showed the following results:

TABLE X: Incidence and location of spontaneous endocrine tumors in Sprague-Dawley rats surviving for more than 2-years

Sex	Effective No. of animals	No. of tumor-bearing animals	Thyroid Medullary carcinoma
Male	42	36(86)	33(79)*
Female	39	28(72)	19(49)

*Numbers in parentheses indicate percentage (%)

Suzuki et al (1979) shows a high incidence of medullary thyroid carcinomas in Sprague-Dawley rats.

In "An Addendum To a lifetime feeding study of Glyphosate in Rats" Rick B. Oleson. Senior Product Toxicology Specialist of Monsanto, states in a letter of 12/7/82 that "I feel it is most important for an accurate evaluation of the oncogenic response in the thyroid that one should not compare the incidence of animals bearing only C-cell carcinoma. but one should instead compare the combined incidence of animals bearing either a C-cell adenoma or carcinoma".

In the same submission, two pathologists from Experimental Pathology Laboratories, Martin G. Robl, D.V.M., Ph.D. and William E. Ribelin, D.V.M., Ph.D. (Senior Pathologist) have addressed the issue of C-cell carcinoma. Their letters to F.B. Oleson of Monsanto are attached.

Reevaluation of the Bio/dynamics report (BDN-77-416; 1/7/81) shows that only one dose level is of concern (high-dose) in only one sex (females).

Dr. Kasza recommended that the thyroid slides be reevaluated by Dr. Capen, EPA consultant pathologist. Laurence Chitlik arranged with Dr. Rick Oleson of Monsanto to have the slides foreward to EPA and then via Dynamac to Dr. Capen in order to obtain an additional evaluation.

Conclusions:

Although the thyroid slides are being reevaluated by Dr. Capen, considering the reviewed data, literature sources, other pathologists opinions, and the variations in the number of C-cell carcinomas in control groups under identical conditions (Table IV), we agree that thyroid C-cell adenomas and C-Cell carcinomas should be combined to establish oncogenicity. Based on the incidence of combined adenomas and carcinomas which is not statistically significant as compared to the control incidence, our tentative conclusion is that Glyphosate is not oncogenic in the lifetime feeding study in rats.

William Dykstra FDC 2/10/83

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I concur with the above pathology assessment and tentative conclusions.

Louis Kasza

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REFERENCES

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2. J.D. Burek; PATHOLOGY OF AGING RATS (1978); CRC Press, Inc.; page 33

3. A LIFETIME FEEDING STUDY OF GLYPHOSATE IN RATS; BDN-77-416; 1/7/81

4. AN ADDENDUM TO A LIFETIME FEEDING STUDY OF GLYPHOSATE IN RATS: Special Report MSL-2009; 1/26/83

5. H. Suzuki, U. Mohr, and G. Kimmerle, SPONTANEOUS ENDOCRINE TUMORS IN SPRAGUE-DAWLEY Rats; (1979); J. Cancer Res. Clin. Oncol. 95. 187-1961

6. Thompson, S.W. and R.D. Hunt; SPONTANEOUS TUMORS IN THE SPRAGUE-DAWLEY RAT: Incidence rates some types of neoplasms as determined by serial section versus single section technics (1963) Ann. N.Y. Acad. Sci. 108:832-845.

7. MacKenzie, W.F. and F.M. Garner; COMPARISON OF NEOPLASMS IN SIX SOURCES OF RATS: (1973); J. Natl. Cancer Institute 50:1243-1257.

Attachments

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