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

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OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Peer Review of Parathion

From: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 8/1/86
Mission Support Staff
Toxicology Branch/HED (TS-769C)

To: Ed Allen
Product Manager #12
Registration Division (TS-767C)

The Toxicology Branch Peer Review Committee met on July 1, 1986 to discuss and evaluate the data base on Parathion (ethyl parathion). Particular attention was focused on the oncogenic potential of the chemical toward Osborne-Mendel rats.

A. Individuals in Attendance:

1. Peer Review Committee: (signatures indicate concurrence with the peer review unless otherwise stated).

Theodore Farber — Theodore M. Farber

William Burnam — Wm D Burnam

Anne Barton — Anne Barton

Reto Engler — Reto Engler

Louis Kasza — Louis Kasza

Bertram Litt — Bertram Litt

Esther Rinde — Esther Rinde

Judith Hauswirth — Judith W. Hauswirth

Robert Beliles

2. Scientific Reviewers: (non-Committee members responsible for presentation of data; signatures indicate technical accuracy of panel report).

Robert Zendzian

George Ghali — G. Ghali

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3. Peer Review Committee Members in Absentia: (Committee members who were not able to attend the discussion; signature indicate concurrence with the overall conclusions of the Committee).

Stephen Johnson

[illegible]

John A. Quest

Richard Hill

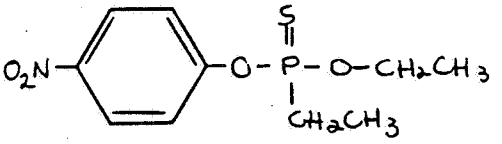
B. Material Reviewed:

The material available for review consisted of the toxicology chapter of the registration standard for parathion and DER's of the NCI and Bio/dynamics chronic rat studies and of the NCI mouse oncogenicity study and a memorandum of 4/24/86 by Robert Zendzian on the rereading of thyroid slides for the Bio/dynamics chronic rat study.

C. Overview of Toxicology Issues:

Parathion is an organophosphorus pesticide which is used on a wide variety of fruit and nut trees, berries, vegetables, field crops and ornamental plants. Several toxicological issues, in addition to oncogenicity, are linked to parathion. These include its extreme acute toxicity typical of cholinergic poisoning and retinal atrophy and sciatic nerve degeneration observed in rats after chronic exposure.

Structure:



Parathion
(O,O-diethyl O-4-nitrophenyl phosphorathioate)

D. Evaluation of the Evidence:

1. NCI Mouse Oncogenicity Study of Parathion

a. Discussion of the Study

B6C3F$_1$ mice were exposed to Parathion (99.5% purity) in the diet at levels of 80 and 160 ppm. Male mice were exposed for 71 and 62 weeks to the low and high dose diets, respectively; female mice were exposed for 80 weeks. Mice were then placed on control diets until termination (90 weeks). Fifty mice of each sex were assigned to each dose level; however, only 10 matched controls/sex were included since the NCI used "pooled" controls for statistical comparison purposes.

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Abdominal distention, tremors and alopecia were observed in all treated groups, males and females. Males also exhibited rough hair coat, diarrhea and hyperexcitability. Body weight gain was decreased in a dose-related manner in male but not female mice and returned to normal after mice were taken off the test diet. Mortality could have been affected in the high dose male mice but because of the limited number of matched controls, this was difficult to determine.

b. Study Evaluation and MTD Consideration:

As evidenced by the dose-related decreased body weight gain, the MTD was reached in male mice; however, available evidence indicates that it was probably not reached in female mice.

This study is flawed in that 1) only two doses were used; 2) the concurrent control contained only 10 mice per sex; 3) some tissues were not examined microscopically; 4) some male mice were dosed only for 62 weeks instead of the usual 80 weeks; and 5) concerns have been raised in audits of the test facility (Gulf South Research Institute).

2. NCI Rat Oncogenicity Study of Parathion

a. Discussion of Study

Osborne-Mendel rats were exposed to Parathion (99.5 % purity) in the diet at time-weighted average dose levels of 32 and 63 ppm for male rats and 23 and 45 ppm for female rats. Fifty rats/sex were used for the dosed groups but only 10 rats/sex were used in the matched control group.

Due to excessive toxicity seen in male rats at the original dosage levels of 40 and 80 ppm these dosages were changed to 30 and 60 ppm after week 13 of the study. The original dosages (20 and 40 ppm) for females were also increased at this time to 30 and 60 ppm; however, this level was too high and the dosages were returned to 20 and 40 ppm at week 46.

A depression of body weight gain remained at approximately 10% for male rats while they were on test diets despite lowering the dose levels at week 13 of the study. Raising the dosage levels for female rats resulted in a depression of body weight gain of 10-15%. Weight gain returned to control levels once both male and female rats were removed from the test diets.

There was no difference in survival between the control and dosed groups. Treatment related clinical signs observed in both male and female rats were tremors, hyperexcitability and hyperactivity.

The incidence of pertinent neoplastic lesions seen in this study is summarized in the following table.

Neoplastic Lesions Seen in Rats Fed Parathion

Organ/Neoplasm	Pooled Controls	Matched Controls	Low Dose	High Dose
		Males		
Adrenal				
Cortical adenoma	2/80(2.50)[1]	0/9(0)	5/49(10.20)	9/46(19.56)
Cortical carcinoma	1/80(1.25)	0/9(0)	2/49(4.08)	2/46(4.35)
Cortical adenoma + carcinoma	3/80(3.75)	0/9(0)	7/49(14.28)	11/46(23.91)
Pheochromocytoma	-	0/9(0)	2/49(4.08)	2/46(4.35)
Thyroid				
Follicular-cell adenoma	5/76(6.57)	3/10(30.0)	2/46(4.35)	8/43(18.60)
C-cell carcinoma	0/76(0)	0/10(0)	1/46(2.17)	1/43(2.33)
Pancreatic Islets				
Islet-cell carcinoma	0/79(0)	0/9(0)	1/49(2.04)	3/46(6.52)
Pituitary				
Adenoma	21/72(29.17)	4/9(44.44)	10/42(23.81)	13/43(30.23)
Adrenal				
Cortical adenoma	4/78(5.13)	1/10(10.00)	4/47(8.51)	11/42(26.19)
Cortical carcinoma	0/78(0)	0/10(0)	2/47(4.25)	2/42(4.76)
Cortical adenoma + carcinoma	4/78(5.13)	1/10(10.00)	6/47(12.76)	13/42(30.95)
Pheochromocytoma	-	1/10(10.00)	2/47(4.25)	2/42(4.76)
Thyroid				
Follicular-cell adenoma	3/80(3.75)	1/10(10.00)	4/45(8.89)	1/43(2.32)
C-cell carcinoma	7/80(8.75)	2/10(20.00)	2/45(4.44)	3/43(6.98)
Mammary Gland				
Fibroadenoma	9/85(10.59)	2/10(20.00)	16/50(32.00)	8/50(16.00)

[1] The number in parenthesis is the percent incidence.

Statistical significance was reached for the following tumor types:

For Males:

1. A significant linear trend ($p \leq 0.001$) for adrenal cortical adenomas and adrenal cortical adenomas plus carcinomas when comparing dosed groups with pooled controls;
2. A significant difference ($p \leq 0.05$) for adrenal cortical adenomas and pancreatic islet-cell carcinomas in the high dose groups, adrenal cortical adenomas plus carcinomas for the low dose group and thyroid follicular-cell adenomas in the high and mid dose group compared to the pooled controls;
3. A significant linear trend ($p \leq 0.05$) for combined adrenal cortical adenomas and carcinomas and thyroid follicular-cell adenomas when comparing dose groups with matched controls;
4. A significant difference ($p \leq 0.001$) between the high dose group and pooled controls for combined adrenal cortical adenomas and carcinomas; and
5. A significant linear trend ($p \leq 0.05$) for thyroid follicular-cell adenomas and pancreatic cell carcinomas when comparing dosed groups with pooled controls.

For Females:

1. A significant linear trend ($p \leq 0.05$) for adrenal cortical adenomas and combined adrenal adenomas and carcinomas when comparing dosed groups with pooled and matched controls;
2. A significant difference ($p \leq 0.001$) for adrenal cortical adenomas and combined adrenal cortical adenomas and carcinomas between the high dose group and pooled controls; and
3. A significant difference ($p \leq 0.05$) for mammary gland fibroadenomas between the low dose group and the pooled controls.

The NTP report concludes, "In the male and female Osborne-Mendel rats receiving parathion in their diet, there was a higher incidence of cortical tumors of the adrenal in pooled or historical controls, suggesting that parathion is carcinogenic to this strain of rat".

Historical control data on this strain of rat, derived from the NCI's Carcinogenesis Testing Program was available to the Committee (Goodman et al. Toxicology and Applied Pharmacology 55, 433-447, 1980).

Neoplasms in Control Osborne-Mendel Rats

Site	Tumor Type	Number of Rats (%) Of 975 Males	Of 970 Females
Adrenal gland	cortical adenoma	17 (1.7)	33 (3.4)
	cortical carcinoma	8 (0.8)	3 (0.3)
Thyroid gland	follicular-cell adenoma	42 (4.3)	18 (1.9)
	follicular-cell carcinoma	27 (2.8)	15 (1.5)
Pancreatic islet	islet-cell adenoma	1 (0.1)	0
Mammary gland	fibroadenoma	13 (1.3)	213 (22.0)

10 Study Evaluation and MTD Consideration:

The MTD was reached in this study in both male and female rats as evidenced by a 10% or greater depression in body weight gain and by physical signs of cholinergic-related toxicity.

This study, like the mouse study, was flawed due to 1) only two dose groups were used; 2) only 10 rats/sex were used in the control group; 3) the test material was fed to rats for only 80 weeks of the 112 week study and 4) lab audit of the study indicated non-adherance to GLP's by the conducting laboratory (Gulf South Research Institute).

3. Oncogenicity Study of Parathion in Sprague-Dawley CD Rats Conducted by Bio/dynamics:

a. Study Discussion

Sixty male and sixty female Sprague-Dawley rats were maintained containing 0, 0.5, 5.0 and 50.0 ppm Parathion (95.11% purity) for 110 and 120 weeks, respectively.

In the males excessive mortality occurred in the 5 ppm group between months seven and 23 but total mortality was comparable in all groups at termination. Weight gain was depressed in both sexes in the 50 ppm dose group. The mean body weights were approximately 16 and 18 percent lower than controls in high dose males and females, respectively at termination. Food consumption was significantly increased in the high dose animals.

Tremors were noted in the high dose male and female rats, frequently at the beginning of the study and sporadically thereafter. Starting at week 21, several high-dose females exhibited an abnormal gait in the hind limbs.

In addition the following toxicity was seen 1) depression in all RBC parameters (hemoglobin and hematocrit) measured in the high dose group, males and females; 2) depression of brain cholinesterase activity in males and females of the high dose group and plasma cholinesterase activity in both males and females of the mid and high dose groups in the high dose group; 3) an increase in retinal atrophy in the high dose female rats; 4) increased loss and/or degeneration of myelinated nerve fibers in the sciatic nerve and its branches for which a clear-cut NOEL could not be determined; and 5) significantly increased brain to body weight ratio in male and female rats.

The incidence of pertinent neoplastic and non-neoplastic lesions found in this study can be found in the following table.

Incidence of Pertinent Neoplastic and Non-neoplastic Lesion in Sprague-Dawley Rats fed Parathion in the Diet

	Dose Level (ppm)			
		Males		
Organ/Lesion	0	0.5	5.0	50.0
Adrenal				
Cortical adenoma	25/60(41.67)[1]	30/59(50.85)	23/58(39.65)	27/59(45.76)
Cortical carcinoma	3/60(5.00)	3/59(5.08)	3/58(5.17)	0/59(0)
Thyroid				
Follicular adenoma	1/59(1.69)	1/58(1.72)	2/58(3.45)	5/58(8.62)
Follicular papillary carcinomas	0/59(0)	0/58(0)	0/58(0)	0/58(0)
Hypertrophy/ hyperplasia	1/59(1.69)	3/58(5.17)	3/58(5.17)	0/59(0)
Brain				
Glioma	0/55(0)	3/55(5.45)	1/55(1.82)	0/55(0)
		Females		
Adrenal				
Cortical adenoma	41/60(68.33)	35/59(59.32)	36/59(61.01)	24/58(41.38)
Cortical carcinomas	7/60(11.67)	4/59(6.78)	4/59(6.78)	3/58(5.17)
Thyroid				
Follicular adenoma	1/59(1.69)	0/57(0)	0/57(0)	0/56(0)
Follicular papillary carcinoma	0/59(0)	0/57(0)	2/57(3.51)	0/56(0)
Hypertrophy/ hyperplasia	0/59(0)	0/57(0)	0/57(0)	0/58(0)
Brain				
Glioma	0/55(0)	1/54(1.85)	1/53(1.89)	2/55(3.64)

[1] The number in parentheses represents the percentage incidence.

There was an increased incidence of thyroid follicular adenomas in the high dose male rats (1.69% in the control versus 8.62% in the high dose

group). The low and mid dose group male rats had a slightly higher incidence of thyroid hypertrophy/hyperplasia when compared to the control group. In addition gliomas (brain), which are relatively rare tumors, were seen at a low incidence in the low and mid dose male rats and all dosage groups of female rats.

The thyroid slides from this study were reread by another pathologist. The results are found in the following table.

Thyroid Tumors/Lesions in
the Parathion Study in Male Rats

Tumor/Lesion	Dose (ppm)			
	0	0.5	5.0	50.0
Follicular cell adenoma	0/60 (0)[1]	1/60(1.60)	1/59(1.70)	4/59(6.80)
Follicular cell carcinomas	0/60(0)	0/60(0)	0/59(0)	0/59(0)
Cystic follicular hyperplasia	1/60(1.60)	2/60(3.33)	2/59(3.40)	1/59(1.70)
Diffuse or focal follicular hyperplasia	6/60(10.00)	2/60(3.33)	4/59(6.80)	2/59(3.40)

[1] The number in parentheses indicates the percentage incidence.

The Registrant provided a table of historical control data on the strain of rats used from Bio/dynamics. Data was provided on 14 studies having a total of 1163 male rats "sacrificed post 12 month" and examined, which showed a total of 45 polymorphofollicular/follicular adenoma/papillary adenoma/adenoma". The incidence ranged from 0-8.0% with a mean of 3.9%.

b. Study Evaluation and MTD Consideration

This was an adequately performed oncogenicity study. As evidenced by a 16% and 18% body weight depression in male and female rats, respectively, signs of cholinergic toxicity, depressed hematocrit and hemoglobin values, increased incidence of retinal atrophy in female rats, increased brain to body weight ratio and increase loss and/or degeneration of myelinated nerve fibers in the sciatic nerve, the MTD was slightly exceeded at the high dose in this study.

E. Additional Toxicity Data:

1. Reproductive and Developmental Toxicity:

Parathion was not teratogenic to rats up to 1.5 mg/kg nor to rabbits

up to 16.0 mg/kg. Fetotoxicity was seen in the rabbit at 4.0 and 16.0 mg/kg as shown by an increased number of resorption sites, accompanied by a decrease in the number of live fetuses and percent implantation. Parathion was not fetotoxic to the rat at 1.5 mg/kg. A reliable study for the assessment of the reproductive effects of Parathion was not available for review.

2. Mutagenicity:

Only one acceptable assay was available for consideration, an unscheduled DNA synthesis assay in human WI-38 cells. This assay was positive for induction of repair at dose levels of 10^{-5}M and 10^{-6}M Parathion. A dominant lethal assay in mice, the Ames Salmonella assay, a reverse mutation assay in E. coli, mitotic recombination in yeast and a DNA repair test in E. coli and B. subtilis were all negative but the assays as performed were considered inadequate.

3. Metabolism:

Parathion is rapidly metabolized in the liver to its oxygen analog, paraoxon. Paraoxon is known to be about ten times more acutely toxic than Parathion, itself, and a few orders of magnitude more potent as a cholinesterase inhibitor.

p-Nitrophenol could be produced from the hydrolytic cleavage of the ester bond and subsequently p-aminophenol from the reduction of the nitro group.

4. Structure Activity Information:

Limited structure activity information was made available. It was postulated that p-nitrophenol could be a metabolite of Parathion. Chemicals of this type have been frequently mentioned in the open literature associated with mutagenic and/or oncogenic activity.

F. Weight of Evidence Consideration:

The Committee considered the following facts regarding the toxicology data on Parathion to be important in a weight-of-the-evidence determination of oncogenic poential.

1. Parathion was not oncogenic in a study (NCI) using B6C3F_1 mice; however, this study was also flawed for the reasons listed elsewhere in this report (Section E.1.).

2. In a study conducted for the NCI, Parathion induced a statistically significant increase in adrenal cortical tumors (adenomas plus carcinomas) in the low and high dose male, Osborne-Mendel rats and in the high dose females when compared to the pooled controls. A statistically significant trend was also found in male rats for thyroid follicular cell adenomas and pancreatic cell carcinomas when comparing dosed groups with pooled controls. This study was flawed for the reasons listed elsewhere in this report (Section E.2.).

3. Historical control data on Osborne-Mendel rats obtained from the NCI Carcinogenesis Testing Program indicated that the percent incidence of adrenal

cortical tumors in this study was well above (6-8x) the average incidence of the historical controls for both male and female rats, the percentage incidence of thyroid adenomas in males of this study was approximately 4 times that of the historical controls, and the percentage incidence of pancreatic islet-cell carcinomas in males of this study was approximately 60 fold greater than the historical controls.

4. The MTD was reached in the NCI rat study as evidenced by a 10% or greater depression in body weight gain and by physical signs of cholinergic-related toxicity.

5. In a well-conducted study (Bio/dynamics) in Sprague-Dawley rats, Parathion induced an increased incidence of thyroid follicular cell adenomas in male rats. A rereading of the thyroid slides from this study resulted in one less thyroid adenoma diagnosed (5/58, first reading; 4/59 second reading).

6. Historical control data provided by Bio/dynamics indicated that the incidence of thyroid follicular cell adenomas was just outside the historical range (8.62% vs 0-8.0%) for the original diagnosis and within the historical control range (6.8% vs 0-8.0%) after the second reading of the thyroid slides.

7. The MTD was probably slightly exceeded in Sprague-Dawley rats at the high dose in this study as evidenced by a 16% and 18% body weight depression in males and females, respectively, signs of cholinergic toxicity, depressed hematocrit and hemoglobin values, increased incidence of retinal atrophy in female rats, increased brain to body weight ratio and increased loss and/or degeneration of myelinated nerve fibers in the sciatic nerve. However, since this was a combined chronic/oncogenicity study, the high dose was appropriate for chronic toxicity.

8. Parathion was not teratogenic to rats or rabbits and did not affect reproduction in rats. A chronic dog study was not availabale for evaluation.

9. Parathion was positive for DNA repair in human WI-38 cells. This assay was conducted in an adequate manner and was considered acceptable. Parathion was negative in several other assays for mutagenicity (including the Ames Salmonella assay and domnant lethal assay in mice), but these were all considered to be unacceptable.

10. Parathion could be metabolized to p-nitrophenol which belongs to a class of chemicals that are associated with mutagenic and/or oncogenic activity.

G. Classification Oncogenic Potential:

The Committee concluded based upon the available evidence that Parathion meets the criteria of a category C oncogen. The NCI study on parathion did not specifically or exactly meet the verbiage used in any of the four criteria of category C; however, it meets a combination of criteria (a) definitive malignant tumor response in a single well-conducted experiment and criteria (b) marginal tumor response in studies having an inadequate design or reporting. Parathion produced a definitive tumor response at one sight and a marginal response at two sites in an inadequately designed study. The weight-of-evidence did not meet any of the criteria listed as "sufficient evidence" of carcinogenicity which would categorize it as a B-2 oncogen.

Results from the NCI study, indicated an increased incidence of adrenal cortical tumors in male and female Osborne-Mendel rats and to a lesser degree an increase in thyroid follicular cell adenomas and pancreatic islet-cell carcinomas in male Osborne-Mendel rats. The second rat study conducted with Parathion using Sprague-Dawley rats was considered by the Committee to be negative for oncogenicity. The initial review of the study indicated an increased incidence of follicular cell adenomas of the thyroid gland in the high dose males. A re-reading of the slides showed one less high dose tumor and no accompanying hyperplasia. Historical control data showed the high dose effect to be within the historical control range. The mouse study conducted on Parathion was negative but was not considered by the Committee to be adequate for judging the oncogenic potential of Parathion in the mouse. The Committee recommended that another oncogenicity study in the mouse be required.

The Committee felt that the weight-of-the-evidence did not warrant a quantitative estimation of the oncogenic potential of Parathion because of the deficiencies of the NCI study and the negative results for oncogenicity obtained in another strain of the same species. Although the Committee decided that a repeat study in the Osborne-Mendel rat should not be required at this time, they thought that the results of such a study would be a better indicator of the oncogenicity of Parathion in the rat. Emphasis would be placed on the results of this study along with a repeat mouse study for the determination of the oncogenic potential of Parathion.

Moreover, the Committee noted that additional and adequate short-term (mutagenicity) tests are required on Parathion. The results of these tests will be valuable in further evaluations on Parathion.

The Committee considered whether the results of the Osborne-Mendel rat study indicated that Parathion possessed hormonal activity, since adrenal cortical tumors were induced by Parathion in this study and to a lesser extent pancreatic islet-cell and thyroid follicular-cell tumors. Since Parathion does not belong to a class of compounds known to have hormonal activity, a secondary mechanism for this effect was postulated. However, the Committee concurred that Parathion was exerting some yet unknown effect on the endocrine system.