8-1-86

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

MEMORANDUM

SUBJECT: Parathion, Special Studies on the Eye and the Sciatic Nerve

TO: Ed Allen PM-12
Registration Division (TS-767)

FROM: Robert P. Zendzian PhD 5/86
Pharmacologist
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HED (TS-769)

THROUGH: Reto Engler PhD, Head
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Theodore M. Farber PhD, Chief
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Compound; Parathion — Tox Chem #637

Registration #524-27 — Registrant; Monsanto

Accession #263986 & 263987 — Tox Project #2190

Action Requested

Review the following submissions.

1) Electroretinographic evaluation, G.A. Edwards, Monsanto, R.D. No. 693, July 23, 1986, Accession # 263986

2) Sciatic nerve evaluations, G.A. Edwards, Monsanto, R.D. No. 693, July 23, 1986, Accession # 263987

Conclusion and Recommendation

1) Electroretinographic evaluation

Statistically significant depression of the amplitude of the b-wave of the ERG of female rats dosed for two years at 8 and 32 ppm was observed. The b-wave of females dosed at 2 ppm and males dosed at 2, 8 and 32 ppm also appeared to be depressed but the large individual variation in b-wave amplitude

made evaluation impossible. Focal posterior subcapsular cataract and choridal pallor also appeared to be increased in a dose-related manner but the conditions of the study make it impossible to be certain of these effects.

2) Sciatic nerve evaluations

Comparison of the item submitted with the origional submission shows that most of the material appears to be a resubmission of the origional data in a different format[1]. It is impossible to determine what, if any, is new data. The submission could not be evaluated.

Recommendations

1) Electroretinographic evaluations

It is recommended that the registrant proceed with developing an electroretinographic study of the acute effects of parathion in the rat. Such a study has been recommended by Toxicology Branch. In designing and performing this study attention must be given to recording technique in order to minimize experimental interanimal variation.

2) Sciatic nerve evaluations

It is recommended that the Registrant revise this report so that it clearly indicates what evaluations were performed subsiquent to the first report. The new report should reference the first report (3/24/83) and state that this is a report of additional pathological determinations. The registrant may wish to discuss this matter with the Agency before producing the new report.

Background

A chronic feeding study of parathion in the rat, submitted in 1984, showed a compound-related degeneration of the retina. This toxic effect was demonstrated by direct observation and by histopathology. Based on this observation the Agency requested that the Registrants perform electroretinographic (ERG) evaluation of rats treated with parathion. The Registrant identified a chronic feeding study in rats by Bayer-Cheminova that was due to be completed in early 1986 and arrainged for Dr. Lionel Rubin V.M.D. to perform ERG evaluations on rats from this study. The first document submitted is Dr. Rubin's report of the ERG evaluation.

In the same 1984 chronic feeding study a special histological evaluation showed degeneration in the sciatic nerve at the high dose. However this evaluation was not performed at the low and intermediate doses and it was imposible to determine

1. A two-year chronic feeding study of ethyl parathion in rats Neurotoxzicity Evaluation, Experimental Pathology Laboratories, March 24, 1983

if a NOEL was demonstrated. The second document submitted is this histological evaluation.

Discussion

1) Electroretinographic evaluations

There are several problems associated with the evaluation and interpertation of this study. First, we have no information on the feeding study in which the rats were included other then the statement of dose and duration (0, 2, 8 & 32 ppm for two years). The report of the study will not be available for at least a year. At this time we have no information on what toxic effects were observed in this study. In 1984 we received a report of a two-year rat feeding study. The doses used in that study were 0, 1, 5, and 50 ppm. A spectrum of toxic effects were reported at 50 ppm which included retinal degeneration, observed directly and by histopathology, and cholinesterase inhibition. At the next lower dose, 5 ppm, only cholinesterase inhibition in a single sex was reported. Thus, we have no direct comparison by dose to make an estimation of the toxicity of the maximum dose tested in this study, 32 ppm.

The most impressive item in this study is the wide range of individual variation recorded in the ERGs. Considering b-wave amplitude in uV, the means and SD are 98 $\pm$ 46 and 101 $\pm$ 59 for the control males and females respectively. Also the polarity of the recording was either positive or negative because of the "random placement of the recording electrode". We cannot say whether the variation reported is intrinsic or is due to the conditions under which each recording was taken or a combination of both. No doubt the individual variation, whatever its source, will act to obscure any compound-related effects.

Dispite these problems I believe that there is a compound-related depression of the amplitude of the b-wave in the females at 8 and 32 ppm. In the previously submitted study the females were the most sensitive sex in that they showed effects on the eyes and the males did not. Considering the mean b-wave amplitude as percent of control there could be a compound-related depression in the females at 2 ppm and in the males at all doses. However, the individual variation in each group makes it impossible to verify this possibilty.

The opthalmoscopic observations are of necessity somewhat subjective and hard to quantitate but there does appear to be a compound-related increase in the percent of eyes examined showing focal posterior subcapsular cataract and choroidal pallor. Organophosphate cholinesterase inhibitors, applied topically for glacoma, have been shown to produce cataract in human patients. Thus the observation of cataract in this

study is not a new observation for this class of compounds and could be compound-related.

Dr. Rubin considered that the choroidal pallor may be due to decreased circulation in the choroidal layer of the retina. A small degree of cholinesterase inhibition in the end arteriols to this area could result in increased tonal constriction of the vessels and a decreased circulation to the area. This would agree with his observation of 'retinal vessels narrow' in one female at 32 ppm and 'vessels in choroid narrowed' in two females at 32 ppm.

2) Sciatic nerve evaluations

Experimental Pathology Laboratories Inc. performed a special evaluation of the sciatic nerve from rats on a chronic feeding study of ethyl parathion (Biodynamics Project Number M-6, 77-2055). In this evluation EPL performed a "detailed qualitative and quantiative neuropathologic examination made on ten control (five of each sex) and ten high dose animals (five of each sex), as well as a qualitative examination made on ten low and ten mid dose rats." The quantitavive evaluation, performed only on control and high dose animals, showed significant compound-related effects. However, since this evaluation was not made on low and mid dose animals a No Observable Effect Level (NOEL) could not be determined. Toxicology Branch recommended that the evaluation be extended to all of the doses. The new pathology submision by Experimental Pathology Laboratories (July 2, 1986) is extremely confusing. No reference is made to the first report. The introduction and methods section appears to be a repeat of the first submission. The 'Summary Incidence Table' (V-23 through V-28) is a partial presentation of data from the first report in a different form. The 'Quantitative Histological Measurements of Sciatic Nerves from One Micron Epon Section' appears to be new data but it is not clear what or how.

The report as submitted cannot be evaluated.

Data Evaluation Report

Compound Parathion

Citation

Electroretinographic evaluation, G.A. Edwards, Monsanto, R.D. No. 693, July 23, 1986, Accession # 263986

Reviewed by Robert P. Hendzian PhD
Pharmacologist

Core clasification Acceptable

Conclusion

Statistically significant depression of the amplitude of the b-wave of the ERG of female rats dosed for two years at 8 and 32 ppm was observed. The b-wave of females dosed at 2 ppm and males dosed at 2, 8 and 32 ppm also appeared to be depressed but the large individual variation in b-wave amplitude made evaluation impossible. Focal posterior subcapsular cataract and choridal pallor also appeared to be increased in a dose-related manner but the conditions of the study make it impossible to be certain of these effects.

Background

A chronic feeding study of ethyl parathion in rats was conducted by Bayer (T1016770). Rats were dosed at 0, 2, 8 or 32 ppm parathion in the diet for 24 months. The detailed materials, methods and results of this study are not available. Shortly before termination of this study, eletroretinographic (ERG) evaluations and direct ophthalmoscopic examinations were performed on animals from the study. The evaluations were performed by Lionel F. Rubin V.M.D. whose report is included in the document being evaluated.

Materials

Rats that had been dosed with ethylparathion for 24-months at doses of 0, 2, 8 or 32 ppm in the diet.

Methods

Examinations were performed on the following rats;

Dose ppm	# Males	# Females
0	12	10
2	9	11
8	11	10
32	10	10

5

Rats were anesthetized with pentobarbital sodium and the pupils dilated with tropicamide. "Recording needle electrodes were placed subcutaniously lateral to the lateral canthus anterior to the ear and subconjunctivally into the inferior conjunctival fornix. The subconjunctival electrode was placed after instillation of tetracaine solution. A needle electrode for grounding was placed subcutaneously at the occiput. The recording was made without reference to maintaining the same polarity. Some recordings appear to be cornea-negative, while others were cornea-positive, this occurring because of the random placement of the recording electrode at either conjunctiva or skin. Prior to recording the electroretinogram (ERG), the rats were dark adapted for at least 8 hours.

The ERG was recorded in a room illuminated only by dim red light. The ERG was elicited using a single stroboscopic blue-white flash provided by a Grass PS22 photostimulator set at intensity 1 approximately 30 cm from the eye. Recordings were taken from the right eye in all animals. A recording was taken from the left eye as well in rat 197F, a 2 ppm level female, because of the presence of a very low amplitude response from the right eye."

"The ERG was evaluated by qualitative inspection and by measurement of the b-wave parameters. The implicit time of the b-wave was measured from stimulus onset; the b-wave amplitude was measured from the baseline. Because of the presence of a large stimulus artifact distorting the a-wave, the usual measurement of b-wave amplitude from maximum deflection of the a-wave to b-wave peak could not be done. The a-wave appeared to be present in all recordings."

Results

The following table is from page 2 of Dr. Rubin's report.

Animal #	b-wave latency msec	b-wave amplitude uV
Control males		
3M	100	60
9M	100	110
12M	100	140
20M	60	100
21M	60	160
23M	90	50
24M	60	60
29M	80	150
37M#2	70	140
40M	80	30
41M	100	120
mean n=12	81	mean n=12 98

Animal #	b-wave latency msec	b-wave amplitude uV
Control females		
51F	70	120
55F	100	100
56F	90	40
59F	100	140
61F	90	30
75F	90	180
76F	80	150
79F	80	160
93F	80	160
100F	60	10
mean n=10	84	mean n=10 101
2 ppm males		
102M	70	40
109M	90	70
112M	90	80
114M	80	150
119M	90	50
130M#2	90	50
138M	70	160
142M	80	100
148M	60	20
148M#2	70	10
(ERG from rat 115 missing)		
mean n=9	80	mean n=9 79
females		
152F	90	60
161F	60	20
162F	80	50
168F	80	100
173F	80	120
178F	90	60
183F	90	100
188F	80	160
191F#2	80	70
194F	80	50
197F	80	5
197F(left eye)	70	20*
mean n=11	81	mean n=11 72

*not included in calculations

Animal #	b-wave latency msec		b-wave amplitude uV
8 ppm males			
205M	70		25
208M	70		100
212M	60		30
222M	60		140
223M	70		80
225M	80		55
231M	90		160
236M#2	40		20
237M	80		45
243M	80		80
248M	60		25
mean n=11	70	mean n=11	71
8 ppm females			
259F	80		10
266F	70		160
277F	60		20
280F	90		30
281F	80		60
288F	60		30
293F	80		90
294F	80		90
294F#2	80		70
295F	80		50
297F	80		10
297F#2	80		15
mean n=10	74	mean n=10	48
32 ppm males			
302M	70		170
307M	70		40
311M	90		70
334M	90		130
336M	70		50
337M	90		60
342M	80		80
343M	80		50
349M	80		85
mean n=10	80	mean n=10	77

Animal #	b-wave latency msec	b-wave amplitude uV
32 ppm females		
395F	80	60
360F	70	70
362F	70	10
365F	70	20
366F	90	70
371F	80	40
374F#2	70	10
375F	70	55
379F	70	50
400F	70	70
mean n=10	74	mean n=10 45

The major abnormalities seen in the opthalmoscopic examinations were focal posterior subcapsular cataract and choroidal pallor. The incidence of these observations, as total number of eyes showing the effect per total number of eyes observed, is given below.

Focal Posterior Subcapsular Cataract

Dose ppm	Males #ob/#t	%	Females #ob/#t	%
control	5/24	21	0/20	0
2	3/20	15	3/22	14
8	5/22	26	3/20	15
32	9/20	45	4/20	20

Choroidal pallor

Dose ppm	Males #ob/#t	%	Females #ob/#t	%
control	2/24	8	8/20	40
2	4/20	20	9/22	41
8	6/22	27	6/20	20
32	10/20	50	12/20	60

Under his detailed presentation of observations of choroidal pallor Dr. Rubin made special notations on the 32ppm females as follows;

"32 ppm female: 360B**** 371B* 374B 375B** 379B**** 400B**

B both eyes
*retinal vessels narrow
**severe pallor
*** slight pallor
**** vessels in choroid narrowed"

Conclusions

Dr. Rubin concluded as follows;

"The presence of choroidal pallor (in 2 above) may indicate a closure of the small vessels in the choroid and may infer that these vessels are no longer functional because of a underlaying retinal disorder. The presence of relatively normal retinal blood vessels should indicate that the inner retinal layers are still functional, thus implying that the outer layers of the retina are affected. It is also possible that the pallor is secondary to a thickening of the choroidal vascular walls. Were there an outer retinal degeneration, the ERG should be extinguished. There seems to be no correlation between choroidal pallor and low ERG amplitudes in this study, and there does not appear to be a statistically significant dose related relationship to the presence of choroidal pallor.

In summary, there appears to be no ophthalmoscopically visible effect associated with the administration of the test compound in the animals examined. Whether there is an electroretinographic effect is equivocal."

Dr. G.A. Edwards, of Monsanto, provided his own summary of Dr. Rubin's report and concluded as follows;

"Dr. Rubin concluded that there were no visible treatment-related ophthalmological effects in rats as a result of chronic administraton of diets contining up to 32 ppm ethyl parathion and that the electroretinographic effects were equivocal. However, it is my opinion that there was a clear, dose-related decrease in b-wave amplitude in mid (8 ppm) and high-dose (32 ppm) females. Whether or not these decreased amplitudes represent an adverse functional defect is unclear. Further evaluation of the possible chronic ocular effects of ethyl parathion must await the results of the light and electron microscopic pathological examinations."

Dr. Edwards provided a statistical evaluation of the b-wave effects in his table 1 which is presented below.

Table 1

Electroretinographic B-Wave Amplitudes of Rats Fed Diets Containing Ethyl Parathion for Two Years.

Dietary Concentration (ppm)	B-Wave Amplitude[a] (uV)	%control[b]
Males		
0	98 ± 46	100
2	79 ± 49	80
8	69 ± 48	79
32	77 ± 43	79
Females		
0	101 ± 59	100
2	72 ± 45	71
8	48 ± 45*	48
32	46 ± 24*	46

(a) Mean ± SD. Where more than 1 ERG was conducted on a given animal, the average of the values reported was used for that animal.
(b) Values calculated by this reviewer
* $p \leq 0.05$ using Dunnett's test

Discussion

There are several problems associated with the evaluation and interpertation of this study. First, we have no information on the feeding study in which the rats were included other then the statement of dose and duration. The report of the study will not be available for at least a year. At this time we have no information on what toxic effects were observed in this study. In 1984 we received a report of a two-year rat feeding study. The doses used in that study were 0, 1, 5, and 50 ppm. A spectrum of toxic effects were reported at 50 ppm which included retinal degeneration, observed directly and by histopathology, and cholinesterase inhibition. At the next lower dose, 5 ppm, only cholinesterase inhibition in a single sex was reported. Thus, we have no direct comparison by dose to make an estimation of the toxicity of the maximum dose tested in this study, 32 ppm.

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is due to the conditions under which each recording was taken or a combination of both. No doubt the individual variation, whatever its source, will act to obscure any compound-related effects.

Dispite these problems I agree with Dr. Edwards that there is a compound-related depression of the amplitude of the b-wave in the females at 8 and 32 ppm. In the previously submitted study the females were the most sensitive sex in that they showed effects on the eyes and the males did not. Considering the mean b-wave amplitude as percent of control there could be a compound-related depression in the females at 2 ppm and in the males at all doses. However, the individual variation in each group makes it impossible to verify this possibilty.

The opthalmoscopic observations are of necessity somewhat subjective and hard to quantitate but there does appear to be a compound-related increase in the percent of eyes examined showing focal posterior subcapsular cataract and choroidal pallor. Organophosphate cholinesterase inhibitors, applied topically for glacoma, have been shown to produce cataract in human patients. Thus the observation of cataract in this study is not a new observation for this class of compounds and could be compound-related. Dr. Rubin considered that the choroidal pallor may be due to decreaseb circulation in the choroidal layer of the retina. A small degree of cholinesterase inhibition in the end arteriols to this area could result in increased tonal constriction of the vessels and a decreased circulation to the area. This would agree with his observation of 'retinal vessels narrow' in one female at 32 ppm and 'vessels in choroid narrowed' in two females at 32 ppm.

Data Evaluation Report

Compound Parathion

Citation
Ethyl parathion chronic rat (BD-78-5) Sciatic nerve evaluation: B.Y. Cockrell, Experimental Pathology Laboratories, EP-86-67, Inc., July 2, 1986.

Included in; Sciatic Nerve Evaluations, G.A. Edwards, Monsanto, R.D. No. 693, July 23, 1986.
Accession # 263987

Reviewed by Robert P. Zendzian PhD
Pharmacologist

Core classification Unacceptable Report

Conclusions

Materials

Sciatic nerve was obtained from five male and five female rats of the control, low (0.5 ppm) and medium (5 ppm) dose groups of a two-year rat feeding study of ethyl parathion.

Two-year chronic feeding study of ethyl parathion in rats I.W. Daly & G.K. Hogen, Biodynamics Incorporated, Project # DB 78-005, Study #: 77-20-55, Jan 23, 1984.

This document contained, as Appendix K, the following document;

A Two-Year Chronic Feeding Study of Ethyl Parathion in Rats, B.Y. Cockrell, Experimental Pathology Laboratories Inc., Bio/Dynamics Project Number M-6, 77-2055, March 24, 1983.

Discussion

Comparison of the two Experimental Pathology Laboratories Inc. reports shows that some of the results contained in the the first report are repeated in the second without mentioning that they are repeated. The table on page V-23 of the Jan 23rd report appears to be composed from parts of the tables on pages 14 and 15 of the Mar 24th report. This data shows that a significant increase in nerve abnormallities was observed in the high-dose males and females. Fifty preporations were evlauated in the control and high dose groups and only 10 preporations in the low and intermediate groups. Thus one cannot properly compare these test groups and determine if a NOEL for the effect was obtained.