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

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Methyl Parathion (E 605 Methyl). Chronic Toxicological Study on Rats (2 Year Feeding Study). Addendum to Report Nos. 9889 and 12559. EPA ID NO. 4787-4. Project 7-0839. Caswell No. 372.

TO: D. Edwards PM-12
Registration Division (TS-767C)

FROM: K. Clark Swentzel K. Clark Swentzel, 11/5/87
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THRU: Marcia van Gemert, Ph.D. M. van Gemert 11/12/87
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and

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11/13/87

Action Requested

Amendments were submitted by the registrant (A/S Cheminova) in response to the reviewer's (reviewed by Dynamac Corp, February 24, 1986) comments regarding additional data and information that is required to upgrade the subject study. The reviewer indicated that the Core classification was Supplementary for both oncogenicity and chronic toxicity. Deficiencies were noted in reporting the histopathologic findings, clinical observations, ophthalmic examinations, clinical laboratory studies, cholinesterase activity determinations, histopathologic examinations and no quality assurance statement was provided.

Conclusions

The Core-classification for the subject study should be upgraded from Supplementary to Minimum for oncogenicity but should remain Supplementary for chronic toxicity. Oncogenicity can be upgraded because the current submission from the registrant included historical data for spontaneous lesions in the appropriate strains of rat, the number of tissues examined from certain organs and more specific classification of observed thyroid tumors. Numerous deficiencies, which are subsequently noted, remain in the parameters investigated for chronic toxicity.

The potential biological significance of increased incidences of uterine adenocarcinomas, thyroid C-cell adenomas, Leydig cell tumors and pituitary adenomas, observed in treated rats, will subsequently be evaluated via the peer-review process.

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Specific deficiencies noted by the reviewer and the registrant's response to each (if provided) are listed below.

1/ A protocol of the study was not provided.

Response: An English translation of the original protocol, dated January 1977, was provided. The original protocol was presumably written in German since the study was performed by Bayer A G.

Comments: The protocol did not list lung among the organs that were weighed.

2/ No data on analyses of dietary levels, stability or homogeneity of the test compound in the diet were presented.

Response: No data for the homogeneity of the test material/diet mixture were generated for this study. GC analyses were performed at 3-4 month intervals to check nominal concentrations and over a 1-week period, to determine stability. Mean nominal concentrations were 80, 91 and 94% of theoretical for the 2, 10 and 50 ppm dietary concentrations of test compound, respectively. Therefore, based on these analytical data, the actual dosages of MP were 0.08, 0.46 and 2.35 mg/kg/day. The stability test indicated that the 7-day nominal concentrations were 70, 86 and 88% of theoretical, respectively.

Comments: Although the investigator concluded that the diet preparation was stable for 7 days at the mid- and high- concentrations but only 5 days at the low- concentration, the test protocol indicated that the diet was prepared on a weekly basis.

3/ No individual data on animal observations or summary tables thereof were presented in Report No. 9889, therefore, the incidence of the observed signs could not be determined.

Response: Tables for individual clinical findings were submitted. The registrant stated that "the palpable masses reported on the individual clinical observations were only palpated when they became visible."

Comments: The submitted tables are difficult to follow because the results were not presented in chronological order and the findings were represented by code numbers. Presumably, all animals were examined, however, the tables included only those animals that exhibited clinical changes, ie. 17, 7, 13 and 15 female rats and 26, 16, 10 and 52 male rats in the control, low, mid- and high dose groups. respectively.

Based on the registrant's original report, the reviewer indicated that males and females in the high-dose group showed symptoms of cholinesterase inhibition (tremors) during the first 2 study weeks and during weeks 19-21; also, cholinergic symptoms occurred sporadically in the high-dose females after week 81. However, the submitted clinical data tables indicate that cholinergic symptoms

were observed only among high-dose males.
It should be noted that masses are usually detected by palpation before they become visible.

4/ Individual animal observations for gross pathology were recorded, but summary tables for the findings were not presented nor were findings discussed in the report.

Response: Individual as well as summarized gross pathologic observations were submitted. A discussion of these data were not included.

Comments: A discolored uterus with bloody content was observed more frequently among treated rats than controls, however, the increased incidence was not dose-related:

Uterus: bloody content

Controls	2ppm	10ppm	50ppm
2/100	6/50	7/50	4/50

The incidences of other gross observations were not higher among treated rats than in corresponding controls.

5/ Organs and tissues from animals sacrificed at 6 and 12 months were not assessed histologically.

Response: Subsequently, organs of the animals from the interim sacrifices, which were preserved, were processed and examined histologically. Only the livers of rats sacrificed at 6 months were preserved, however, the registrant mentioned that this interim kill was a supplementary investigation that was not required by the guideline. Histologic information from these animals was of little value because livers from only 1,3, 0 and 0 males and 0, 1, 0 and 2 females from the control, low, mid- and high- dosage groups, respectively, were evaluated. Five rats/sex/group were used for the 12-month histopathologic evaluation, with the exception of the low-dose female group from which 4 rats were used. Individual as well as summarized data were presented.

Comment: No compound-related changes were evident from these data.

6/ Summary tables of non-neoplastic findings were not presented in the report.

Response: Summary tables of non-neoplastic findings were submitted.

Comments: The registrant did not explain why only 50 rats/sex were examined from the control group.

The kidney examinations revealed the following differences on the incidence of PAS-positive substances in cortical tubules when sections from treated and control rats were compared:

Kidney: PAS-positive material in cortical tubules

Control		2ppm		10ppm		50ppm	
M	F	M	F	M	F	M	F
2/50	0/50	19/50	9/50	15/50	4/50	0/49	0/50

The reason for the deposition of PAS-positive material (probably carbohydrate) in the cortical tubules of low- and mid- dose rats (especially males) is not readily apparent. The investigator did not discuss this observation.

An increased incidence of ORO-positive substances in the hepatocytoplasm of mid- and high- dose rats (particularly males) was evident from the following data:

Liver: ORO-positive material in hepatocytoplasm

Control		2ppm		10ppm		50ppm	
M	F	M	F	M	F	M	F
2/50	3/50	2/50	1/50	5/50	1/50	16/49	2/49

These data indicate that lipid infiltration was apparent in the hepatocytes of male rats at dietary levels of 10 and 50ppm methyl parathion.

7/ Summary tables for neoplastic lesions did not mention the numbers of tissues examined histologically.

Response: Revised summary tables, which included limited numbers of organs/systems, as well as the numbers of each examined, were submitted. These tables listed circulation system, cutis/subcutis, brain, pituitary gland, adrenals, pancreas, thyroid gland and reticulohistiocytary system for both males and females; parathyroid gland and testicles for males and liver, lung, salivary glands, ovaries and uterus for females. Evidently these particular tables were submitted because they included the organs/systems that were listed in Table 10 (Neoplastic Lesions in Rats Fed E-605 Methyl for 2 Years) of the evaluation.

The registrant explained that bilateral tumors in paired organs were counted as 1 tumor instead of 2, as previously reported, in order to be compatible with the method of counting tumors that was used to derive historical data for the strain of rat used in this study.

The registrant also corrected two errors that occurred in

previous reports:

1) Report No. 12559: there were 0 uterine adenomas in the control group, not 4 (Table 10 of the evaluation already has 0 instead of 4).

2) Report No. 9889: a thyroid tumor in a control male should have been listed as follicular adenocarcinoma instead of adenoma.

Comments: As previously noted, only 50 control rats/sex were examined.

The reviewer had previously determined the number of tissues examined histologically; these numbers were provided in Table 10 of the evaluation.

The tumor counts in the present submission represent only minor changes in the data shown in Table 10 of the evaluation as follows:

Tumor Count

Organ/System	Neoplasm	Table 10	Amendment
Adrenal:	Pheochromocytoma:		
	Male: Control--	8/50	9/50
	High dose-	3/50	2/50
	Cortical cystadenoma:		
	Male: Low-dose--	0/50	1/50
	High-dose-	0/50	1/50
Testes:	Leydig cell tumor:		
	High-dose-	4/50	2/50
Reticuloendothelial:*	Lymphoma:		
	Male: Low-dose-	0	1

*Number examined could not be determined

8/ Previously reported thyroid adenoma counts did not indicate the proportions of tumors that were C-cell and follicular adenomas, therefore, a more specific classification of the thyroid tumors was requested.

Response: The registrant classified the thyroid tumors in this study as follows:

Thyroid Tumors

Males (No. examined)	Control (49)	2ppm (48)	10ppm (46)	50ppm (48)
C-cell adenoma	5	3	2	11
Follicular adenoma	2	1	0	1

Females (No. examined)	(49)	(47)	(50)	(40)
C-cell adenoma	4	5	9	3

The only malignant tumors diagnosed were: 2 control males with follicular adenocarcinoma and 1 high-dose female with C-cell adenocarcinoma.

As a result of re-diagnosis, tumor classification was changed for 2 animals:

1) Control male: change 1 cystadenoma to 1 C-cell adenoma plus 1 follicular adenoma.

2) Low-dose male: change 1 adenoma to 1 C-cell adenoma plus 1 follicular adenoma.

Comments: The increased incidences of C-cell adenoma in high-dose males and mid-dose females, compared to respective control values, were not statistically significant ($p<0.05$, Fisher's exact test).

9/ Historical control data for spontaneous neoplasms in this strain of rat were not presented.

Response: Two reports that are germane to this issue were submitted: 1) A published report entitled Spontaneous Tumors of 2,000 Wistar TNO/W.70 Rats in Two-Year Carcinogenicity Studies (1986). E. Bomhard, E. Karbe and E. Loeser, JEPTO 7:1/2:35-52 and 2) an unpublished report entitled Frequencies of Spontaneous Tumors in Wistar TNO/W.74 Rats in Chronic Toxicological Tests. E. Bomhard, Institute of Toxicology, Bayer AG, February 23, 1982. The first report involved 962 control males and 968 control females from 11 two-year toxicity/carcinogenicity studies performed between 1973 and 1976 with a strain of rat closely related to that in the subject study. The control rats in the second report consisted of 560 rats/sex of the strain used in the subject study from 8 two-year studies performed between 1975 and 1979.

Historical and current (subject study) incidences of C-cell (adenoma), Leydig cell and uterine (carcinoma/adenocarcinoma) tumors are compared as follows:

C-cell adenoma

1/ Historical data

a/ TNO/W.70 rats

Males: mean = 6.5%; range = 0--18.1% (829 examined)
Females: mean =7.8%; range = 2.2--21.2% (845 examined)

b/ TNO/W.74 rats

Males: mean = 2.1%; range = 0--13.3% (533 examined)
Females: mean = 2.3%; range = 0--17.4% (531 examined)

2/ Current Study (TNO/W.74 rats)
(No. examined)

	Control	2ppm	10ppm	50ppm
Males:	10.2%(49)	6.3%(48)	4.3%(46)	22.9%(48)
Females:	8.2%(49)	10.6%(47)	18.0%(50)	7.5%(40)

Leydig cell tumors

1/ Historical data

a/ TNO/W.70 rats

mean = 2.8%; range = 0--10.4% (933 examined)

b/ TNO/W.74 rats

mean = 6.2%; range = 2.0--16.0% (548 examined)

2/ Current study
(No. examined)

Control	2ppm	10ppm	50ppm
2.0%(50)	4.3%(47)	0%(50)	4.0%(50)

Uterine tumors

1/ Historical data

a/ TNO/W.70 rats (carcinoma/adenocarcinoma)

mean = 6.3%; range = 0--14.4% (905 examined)

b/ TNO/W.74 rats (adenocarcinoma)

mean = 7.5%; range = 0--14.6% (535 examined)

c/ Additional incidences of adenocarcinomas in 3 recent (1978, 1979 and 1981) studies with utreated TNO/W.74 rats (No. examined not given):

1978----18.4%
1979----16.0%
1981----20.0%

2/ Current study (adenocarcinomas)
(No. examined)

Control	2ppm	10ppm	50ppm
8.5%(47)	6.0%(50)	18.0%(50)	17.4%(46)

Pituitary adenomas

1/ Historical data

a/ TNO/W.70 rats

Males:	mean = 16.9%;	range = 2.4--37.7% (783 examined)
Females:	mean = 21.9%;	range = 6.7--39.7% (784 examined)

b/ TNO/W.74 rats

Males:	mean = 12.3% ;	range = 4.4--28.3% (506 examined)
Females:	mean = 16.7% ;	range = 8.3--31.8% (491 examined)

2/ Current Study
(No. examined)

	Controls	2ppm	10ppm	50ppm
Males--	21.3%(47)	30.2%(43)	24.4%(45)	8.7%(46)
Females-	14.3%(42)	15.9%(44)	32.7%(49)	7.9%(38)

Comments: The slightly higher incidence of Leydig cell tumors among low- and high- dose rats, compared to controls, in the subject study was well within the range of historical controls.

A statistically significant ($p<0.05$, Fisher's exact test) increase in the incidence of pituitary adenomas was observed in mid-dose females only. The historical data showed that the spontaneous incidence of this lesion is high in both the TNO/W.70 and TNO/W.74 strains and the range of values exceeded the noted increased incidence in treated females, therefore, this increase does not apppear to be biologically significant.

Although the increased incidence of C-cell adenomas in high-dose males and uterine adenocarcinomas in mid- and high- dose females were not statistically significant ($p>0.05$, Fisher's exact test) in comparison to concurrent controls, these incidences were each slightly above the upper end of the range of respective historical values from the cited reports. The incidence of C-cell adenomas in mid-dose females was within the historical range of values, but well above the mean value.

10/ Ophthalmoscopic examinations were not performed.

Response: The registrant stated that "ophthalmoscopic examinations have not been performed because they were not requested by regulations at that time. Due to the rather high incidences of spontaneous alterations in most of the commonly used strains of albinotic laboratory rats, these investigations are generally of limited value, at least if performed at the end of the study (as is required by the current FIFRA-guidelines."

11/ No quality assurance statement was provided.

Response: The registrant indicated that "the study commenced in January 1977, and at that time no GLP-requirements existed for pesticides regulated by FIFRA. Therefore, a formal quality assurance statement, according to the spirit of the regulations enacted in June 1984, cannot be given."

12/ Deficiencies noted by the reviewer that were not addressed by the registrant.

a/ Insufficient numbers of animals were used for clinical laboratory studies and cholinesterase determinations; also, brain cholinesterase activity should have been measured at the 6 and 12 month intervals.

b/ Not all guideline-required tissues (in particular nerve) were evaluated histologically.

c/ Completeness of the histologic examination could not be determined. The reviewer did not explain why.

d/ The gross and histologic exam on controls was incomplete, only 50/100 per sex.

e/ For the pituitary and thyroid, particularly in high dose females, several tissues were lost or not examined (pituitary: 9.6%; thyroid: 3.5%). OECD guidelines recommend that tissue loss should not exceed 10%.

f/ Food efficiency values were not presented. Although not required, these data are useful for evaluating bodyweight changes.

g/ Statistical analysis of relative organ weights was not provided nor was the brain weighed.

h/ The original report indicated that cholinergic symptoms were observed in both treated males and females, however, the data submitted with the amendment showed symptoms in high-dose males only.

Conclusions

The registrant provided the following oncogenic data which is adequate to upgrade the Core-classification for oncogenicity from Supplementary to

Minimum: 1) historical data for spontaneous lesions in Wistar TNO/W.70 and TNO/W.74 rats, the number of tissues that were examined from possible target organs and more specific classification of thyroid tumors observed in this study. Remaining deficiencies for oncogenicity are: 1) not all guideline tissues (including nerve) were examined histologically, 2) only 1/2 of the control rats were examined, 3)several pituitary and thyroid samples were apparently lost and 4) the number of tissues examined were provided only for those tissues in which increased incidences of neoplastic lesions were observed.

The numerous deficiencies that remain for chronic toxicity obviate the possibility of upgrading the Core-classification for this aspect of the subject study. These include inadequate presentation of clinical data, ophthmalmic observations were not performed, insufficient numbers of animals were used for the clinical laboratory studies and cholinesterase determinations (brain cholinesterase activity was not measured at the interim sacrifices), palpation was not done until tumors were visible, food efficiency values were not presented and statistical analysis of relative organ weights was not provided nor was the brain weighed.

Among the neoplastic lesions that occurred at a higher incidence among treated rats compared to controls: pituitary adenoma, Leydig cell tumors, uterine adenocarcinoma and thyroid C-cell adenoma, only the incidences of the latter two exceeded the upper end of the respective historical ranges.

The potential biological significance of the increased incidences of these neoplastic lesions among rats treated with methyl parathion will be evaluated by peer-review.