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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: American Cyanamide Company Response to Malathion Registration Standard

Tox Chem No.: 535
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You will find appended the Tox Branch response to American Cyanamide's June 24, 1988 technical response to the Malathion Registration Standard.

The following constitutes a brief summary of Tox Branch conclusions addressing those particular sections of the Guidelines with which American Cyanamide has expressed some disagreement as to the data requirements.

1. 83-1 Chronic Toxicity - Dog

The Registrant expresses disagreement with the Agency's conclusion that another chronic toxicity study in the dog should be required. The several parameters in this study commented upon by the Registrant are discussed in the attached Tox Branch response. The Tox Branch position remains the same, that another chronic toxicity study in the dog should be required. The Registrant should be advised to consult with Tox Branch on the proposed manner of assessing cholinesterase inhibition before pursuing the study.

2. 83-1 and 83-2 Combined Chronic/Oncogenicity Testing - Rat - Malathion

The Registration Standard assessment for chronic/ oncogenicity testing of malathion in the rat is somewhat complex. The Agency concluded that NCI oncogenicity bioassays in Osborne-Mendel rats (1978) and F344 rats (1979), while satisfying oncogenicity testing requirements, do not satisfy chronic toxicity testing requirements. The Agency also concluded that the American Cyanamide Company's 1981 2-year Sprague-Dawley rat study does not satisfy the oncogenicity or chronic toxicity testing requirements. With respect to the Sprague-Dawley rat study, the Registration Standard requires an independent reexamination of microscopic slides of tissues in order to properly assess the chronic toxicity responses, even though deficiencies in the study appear to preclude its designation as an acceptable chronic toxicity study. Hence, for these reasons the Registration Standard requires another chronic toxicity study in the rat.

The Registrant has responded to this Registration Standard requirement by proposing a one-year chronic toxicity study in the rat. Tox Branch does not consider this to be an acceptable proposal. Guidelines for chronic toxicity testing in the rat specify a 2-year (effectively lifetime) period of dosing. Further, in view of the questionable chronic toxicity findings in the Sprague-Dawley rat study, it is considered more imperative than otherwise that the study be conducted for the full 2-year period. It is also considered necessary in order to obtain definitive cholinesterase data. The Registrant should be advised to discuss with Tox Branch the protocol for obtaining cholinesterase data prior to initiating the study.

The Registration Standard also requires that this malathion study be pursued using the F344 rat and suggests that the study be conducted as a combined chronic/oncogenicity study (p. 121). The oncogenicity component is here regarded as more imperative than previously in view of additional considerations incorporated in the Tox Branch comments on the Registrant's response with respect to the NCI malathion and malaoxon studies.

Hence, Tox Branch recommends that a contemporary 2-year chronic/oncogenicity study of malathion in the F344 rat be required.

3. 83-1 and 83-2 Combined Chronic/Oncogenicity Toxicity - Rat - Malaoxon

The Registration Standard requires both a chronic and an oncogenicity study to be conducted on the malathion metabolite, malaoxon, and indicates that a combined chronic/oncogenicity study will suffice. The standard suggests that the study be conducted using the F344 rat (p. 121).

American Cyanamide disagrees with the Agency's requirement for the study for the reasons that: (1) malathion studies effectively test malaoxon since the latter is the principle metabolite of malathion; (2) an inconsistency exists in requiring a study on a metabolite that is not food residue; and (3) an anticipated metabolism study should provide the Agency's needs for purposes of risk assessment.

The NCI malaoxon oncogenicity study in the F344 rat (1979) is commented upon in the Registration Standard, and in the attached Tox Branch comments to the Registrant's response. By NTP reexamination, this study yielded equivocal (as defined by NTP) evidence of carcinogenicity of thyroid C-cells. There may be other oncogenic responses as well.

As stated in the Registration Standard, the purpose of this requirement is to clarify the oncogenic potential of malaoxon and to provide additional information as to the effects of malaoxon on cholinesterase inhibition (p. 19).

Tox Branch reiterates the recommendation that a combined chronic/oncogenicity study of malaoxon in the F344 rat be required. The Registrant should be advised to consult with TOX Branch the protocol for assessing cholinesterase inhibition in this study.

4. 83-2 Oncogenicity - Mouse - Malathion

The Registration Standard requires submission of a new malathion oncogenicity study in the mouse. The Registrant takes the position that the NCI oncogenicity study of malathion in the B6C3F1 mouse (1978) should be considered acceptable and, hence, requests exemption from the requirement for a new study. The following points are emphasized by the Registrant as reasons disputing the Agency's concerns: (1) combined incidences of hepatic adenomas and adenocarcinomas for all groups, control included, fall within the historical control range; (2) the high dose group incidence of these tumors almost precisely

match the average historical control incidence; (3) low incidence of these tumors in the contemporaneous control; (4) excessively high doses of malathion employed in this study.

While the foregoing arguments do appear valid, the Registration Standard nevertheless concluded that because of study design flaws and the questionable liver findings [i.e., dose-related tumors (P=0.019) and increased incidence of hepatocellular tumors at the high dose (P=.031)], another study in mice is required. Further examination of this study as discussed in the Tox Branch comments to the Registrant's response serves to reinforce the need for this additional study.

5. 84-3 Structural Chromosomal Aberrations, and 84-4 Other Genotoxic Effects

The Registration Standard concluded that additional mutagenicity studies should be submitted. The Registrant responded that data are available and cited EPA Project Report LSU 3493 as containing the studies covered by Sections 84-3 and 84-4.

Tox Branch obtained LSU 3493 as a follow-up to the Registrant's assertion. The malathion studies in the Stanford Research Institute document have been examined in Tox Branch where it was concluded that detailed reviews of the studies would be of little value in view of the obvious study deficiencies with respect to current Guideline requirements. The studies employed insufficient dosages and there was incomplete documentation as to the study control procedures.

The particular studies conducted on malathion which were examined but which were considered to be inadequate as indicated above include: dominant lethal, unscheduled DNA synthesis, reverse gene mutation, differential toxicity (two strains of bacteria) and mitotic crossing over (recombination) in yeast.

Tox Branch thus considers these mutagenicity studies to remain as Registration Standard data requirements.

6. 81-1 thru 81-6 Acute Toxicology Testing: Manufacturing - Use Product

In the case of each of the studies 81-1 thru 81-6 the Registrant claims that manufacturing concentrate is the same as the technical material and, hence, the required tests are satisfied by those conducted on the technical material as

discussed in Table A of the Registration Standard. The Registrant will not conduct these studies.

Tox Branch concurs with the Registrant, assuming that Registration Division affirms that the manufacturing use product and the technical active material are the same.

Comments on Registrant's Response to
Malathion Registration Standard

I. 83-1 Chronic Toxicity - Dog

The Registrant delineates six points of disagreement with the Agency's assessment of the chronic dog assay.

1. Increased liver and kidney weights - Toxicology Branch I (Tox Branch) contends that liver and kidney weights were elevated in both sexes at all dose levels. Hence, for these findings, the NOEL was not identified. The data in question are here reproduced from the Tox Branch review.

Percent of Control Values at Low, Medium and High Doses

	Organ Weight			Organ Weight/ Body Weight			Organ Weight/ Brain Weight		
Kidney	L	M	H	L	M	H	L	M	H
Male	119	125*	147*	120	123*	158*	120	132*	158*
Female	118	124*	155*	115	125*	178*	114	122*	155*
Liver									
Male	109	121	123	111	118	133*	111	129*	132*
Female	140*	131	141*	134*	131*	151*	135*	128	141*

*Denotes statistically significant changes with respect to control.

The Registrant acknowledges statistically significant increases in absolute and relative kidney weight for both sexes at the mid- and high-dose levels, and asserts that in the absence of statistically significant increases at the low dose, the low dose should be identified as a NOEL.

In view of the numerical increases at the low dose, ranging from 14 to 20 percent in excess of the control for the various modes of expression and that these appear to constitute part of an overall dose-response for both sexes, Tox Branch must adhere to the view that a NOEL has not been identified for increased kidney weight. The fact that the increases noted at the low dose were not statistically significant does not justity concluding these effects were not compound related.

The Registrant acknowledges the high dose to be an effect level in both sexes for increased liver weight, but dismisses findings at other dosage levels as compound related, calling the mid dose (125 mg/kg) a NOEL for this effect.

Tox Branch is of the opinion that for males the increases noted for the low and mid doses, being statistically significant at the mid dose on a brain weight basis, are part of a dose-response relationship without a NOEL. Granted, the increase in the low dose was small (9 to 11%), but the likelihood that the increase was compound related is further supported by the larger increases observed at this dose level for females.

Among females, statistically significant increases in liver weight were observed at the low and high doses on the basis of all three modes of expression, with the smaller increases at the mid dose being marginally significant/nonsignificant. There was no clear evidence of a dose response. Tox Branch considers the effects at all doses to be compound related and that a NOEL was not identified for females.

Where kidney and liver weight increases are concerned, the Registrant appears to ascribe to the philosophy that increases in weights of these organs must be both statistically significant and characterized by a dose-response relationship in order to be identified as compound-related effects. It is certainly true that such findings would be most definitive, however, from a conservative standpoint substantial numerical increases at the lowest dose, in concert with a clear dose response and statistically significant increases at the mid and high doses should merit identifying the lowest dose as a biological compound-related effect level (kidney, both sexes; liver, males) and statistically significant increases at the low and high doses without a clear dose response should justify identifying the lowest dose as an effect level (liver, female).

Tox Branch thus reiterates its position enunciated in the review of the study, namely that a NOEL was not identified for increased liver and kidney weights.

2. Elevated Platelet Count

The Registrant acknowledges statistically significant elevations in platelet count for both sexes at the mid- and high-dose levels, but considers the increases at the low dose to be spurious findings. Tox Branch maintains that effects at the low dose should not be dismissed. In males the increased platelet count (20 to 30%) at each time point was part of a dose response and should be viewed as a biologically meaningful effect of dosing. In females, at the 6-week interval, there was no effect on platelet count at the low dose. However, an effect at the low

dose was evident at the 3-month time point and beyond. At 1 year, the increase at the low dose was statistically signiticant and evidently part of a dose response. Hence, in females, a NOEL was not identified for this parameter.

The Registrant furhter indicates that an increased platelet count of this magnitude should not be viewed as toxicologically or biologically meaningful. Tox Branch believes that this is a question open to speculation. The increases in platelet count were consistent in the course of time and dose related, and at least should be viewed as reflecting an alteration in homeostasis. Tox Branch admits not knowing the toxicological significance of this effect, but does not concur that one or more does (do) not exist. Splenomegaly can explain a decrease in platelet count (Merck Manual, p.1159, 1982) and, likewise, a compromise in spleen function could explain an increased platelet count. However, there was no supporting evidence of an effect upon the spleen in this case. As to the possible toxic consequences of elevated platelet count, one could speculate that an enhanced potential may exist for thrombosis formation, particularly in individuals already predisposed to have strokes or other vascular disease.

3. and 4. Creatinine and Blood Urea Nitrogen (BUN)

The Registrant acknowledges that both creatinine and BUN were decreased at all dose levels after 1 year. The Registrant endeavors to establish that while increases in these two parameters can signify compromised kidney function, decreases as seen in the study should not be viewed as toxicologically significant.

Tox Branch agrees that increases in these parameters often denote an adverse effect of the test material on renal clearance. The decreases noted in this case have not been explained. However, the changes were statistically significant in both sexes and represent a consistent departure from the norm. The fact that an explanation for the effect has not been established does not preclude the existence of a toxicologic perturbation. Conservative assessment of such findings requires the identification of these findings as effects of dosing.

5. SGPT

As was true with respect to platelet count, creatinine and BUN, the Registrant indicates that the direction of change in activity of SGPT is most relevant to the identification of the finding as a toxicological response. In this case SGPT activity was decreased while the view is expressed that an increase would more appropriately signify a toxic effect. This logic, of course, is based upon a reasonably well-understood explanation for increases in activity of this enzyme in response to chemical agents. Simply because a decrease in activity may not have a well-understood etiology cannot justify discounting the effect as

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a consequence (possibly adverse) of dosing. The Tox Branch response to the Registrant's position is to be conservative, the same as that enunciated for platelet count, creatinine, and BUN.

6. Cholinesterase

The Registrant acknowledges the absence of a NOEL for malathion with respect to plasma and erythrocyte cholinesterases. Both of these were inhibited to the extent of 20 to 30 percent at the lowest dose, 62.5 mg/kg/day. Brain cholinesterase was not inhibited at any dose tested.

The Registrant asserts that another study in the dog would be pointless since it could not be anticipated to identity a cholinesterase NOEL that would be below or even low enough to approximate that determined in the human study (Moeller and Rider, 1962) upon which the ADI is currently based.

Tox Branch has the following additional comments to offer. There was, in general, a lack of discovery of substantial inhibitory effects on the various cholinesterases and the lack of an appreciable dose-related response. Furthermore, there was little evidence of a cholinergic response, behavioral or otherwise, even though the doses of malathion administered were relatively high.

In this particular 1-year study, dogs were administered malathion via capsules (once daily) at dosage levels of 0, 62.5, 125, and 250 mg/kg/day (considered equivalent to 0, 2500, 5000, and 10,000 ppm via the diet, assuming for the dog that 1 mg/kg/day is equivalent to 40 ppm).

RBC and plasma cholinesterases were inhibited in both sexes, at all doses and at all time points; however, mean plasma cholinesterase values in male dogs reached a steady state at the lowest dose level and earliest time point, i.e., there was no further decrease in activity with either increasing dose or time. This steady state level of activity occurred at about 70 to 77 percent of control values. In females there was more evidence of a dose-related (not time-related) trend, where levels of activity declined with increasing dose from about 78 to about 63 percent of baseline value.

RBC cholinesterase was inhibited in both males and females to essentially the same extent at all doses and at all time points. This steady state level of activity in both sexes under the influence of malathion was about 75 percent of the control activity.

Brain cholinesterase activity as measured in the cerebrum and cerebellum did not appear to be inhibited by malathion in either sex at any dose level, except possibly slightly so in the cerebrum at the highest dose.

Thus, the lack of clinical evidence of a cholinergic response is consistent with the weak effects on the various cholinesterases assayed.

These effects contrast with those seen in the rat following dietary exposures to malathion. Golz and Shaffer (1956), as cited in USEPA (1975) p.71, found in a 2-year rat feeding study that at the lowest dose tested, 500 ppm, there was marked inhibition of RBC cholinesterase. In another similar rat study, Hazelton and Holland (1953), as cited in USEPA (1975) p.71, reported significant inhibition of all three enzymes (erythrocyte, plasma, brain) at all levels of exposure (100, 1000, 5000 ppm) via the diet. In the actual Hazelton and Holland (1973) paper, inhibition of cholinesterase is reported as "slight" at 100 ppm. One might conclude that the dog has a greater resistance to malathion than the rat, but to confirm the possibility the compound should be administered in comparable ways to both species. It is of interest that Hazelton and Holland (1953) also reported a single I.V. dose of malathion (100 mg/kg) in the dog (one dog) resulted in a sharp decline in plasma cholinesterase activity to about zero and in RBC cholinesterase to about 55 percent of baseline. These cholinesterase effects were accompanied by very perfuse salivation. While it may not be proper to compare responses resulting from I.V. administration with those of oral administration, this study certainly reveals the vulnerability of the two cholinesterases in the dog to malathion.

In the chronic dog study, Tox Branch is concerned that under the conditions of administration (capsule, once daily), malathion was not as bioavailable as the equivalent dose might have been if administered fractionally during the course of the day, or if administered by microencapsulation. This is important to the validity of the study for both cholinergic and noncholinergic effects. Toward resolving this concern, the Registrant should be advised, in repeating the chronic dog study, to administer the compound fractionally on a daily basis or by microencapsulation, rather than by single daily doses via capsule. Alternatively, the Registrant may present arguments to justify the single daily capsule dosing regimen as appropriate for malathion, or submit an auxillary dog study which clearly shows equivalent or, no less dramatic, cholinergic responses via multiple daily dosing as compared with equivalent dosing via single daily capsule dosing with malathion.

Curiously, in the human study (Moeller and Rider 1962) where malathion was administered to volunteers via capsules (presumably once/day) the LEL for RBC and plasma cholinesterases was reported as 24 mg/day (equivalent to 0.34 mg/kg/day for a 70 kg man). The 0.34 mg/kg/day dose is considerably less than the lowest dose employed in the dog study, 62.5 mg/kg/day. A close reading of the Moeller and Rider publication does not reveal whether a given dose was administered once a day or fractionally at various time points but most likely was administered daily in a single capsule. There is considerable room for speculation here, but the evidence in hand suggests that dogs were not responding as

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dramatically to malathion as one might anticipate. The dog may be much less susceptible to malathion by the oral route of administration, but Tox Branch would like to see more evidence that the marginal effects seen in the dog study at relatively high doses were not the consequence of the manner of dosing and that malathion has equivalent potency in the dog whether administered orally as a single daily capsule or by frequent dosing in a manner more analogous to dietary exposure scenarios.

In view of the overall discussion and assessment of this chronic dog study, Tox Branch remains of the opinion that a repeat chronic dog study on malathion is necessary.

II 83-1 and 83-2 Chronic/Oncogenicity - Rat - Malathion

The Registrant is correct in acknowledging that the Registration Standard requires an independent reevaluation of microscopic slides of tissues from the 1981 American Cyanamide 2-year study in the Sprague-Dawley rat. The reasons for this reexamination are set forth on pages 14-15 of the Standard. The Registrant indicates that because of the age of this study, a reevaluation would not likely address all concerns, and proposes as an alternative conducting a 1-year chronic toxicity study in the rat, suggesting that such a study should satisfy chronic toxicity testing requirements for malathion in the rat. Tox Branch must adhere to the requirement for a reevaluation of the Sprague-Dawley rat study since this study appears to reveal varied chronic toxic responses to malathion which require resolution. Also, Tox Branch does not consider a 1-year study in the rat to satisfy the requirements for chronic testing in this species. Thus, Tox Branch must also adhere to the requirement for a new 2-year chronic toxicity study in the F344 rat as set forth in the Registration Standard.

The Registrant is correct in asserting that the Registration Standard affirms (page 9) the NCI bioassays in Osborne-Mendel rats and F344 rats as acceptable negative studies for oncogenicity of malathion. The Standard thus implies that additional oncogenicity testing of malathion in the rat is not required. On the other hand, Table A (page 121) of the Registration Standard requires additional chronic toxicity testing in the rodent, specifically the F344 rat, which the Standard goes on to suggest be conducted as a combined chronic/oncogenicity study (page 15). That the study be conducted so as to include the oncogenicity component is supported by the following additional discussion with respect to the quality and findings of the NCI studies.

The Osborne-Mendel and F344 rat studies on malathion were both conducted for the NCI in the mid-1970s, just prior to promulgation of Good Laboratory Practices (GLP) guidelines. The deficiencies in the manner of conduct of the studies and the questionable aspects of oncogenic findings will be evident in the following discussion.

1. Osborne-Mendel Rat Study

The following summation of information as to the design and findings of this study is developed in part from Gross (1984).

In the Osborne-Mendel rat study, NCI initiated testing of malathion using as the matched control group 10 rats/sex, and employed 50 rats/sex/dose group at dosage levels of 8000 and 16,000 ppm. At the beginning of the study, however, 16,000 ppm was evidently too toxic, so a new high-dose group was started at 12,000 ppm, approximately 5 weeks into the study, and to match this group 5 rats/sex were added to the control group giving a total of 15 rats/sex as "matched" controls. Even the 12,000 ppm dose was later considered too toxic, so dosing for low- and high-dose groups was decreased to 4000 and 8000 ppm, respectively, employing the same test animals. Hence, the time-weighted average doses for the duration of the study were 4700 ppm for low-dose animals and 8150 ppm for high-dose animals. It was also the intent of NCI to combine matched control animals for several agents under test, including malathion, for use as pooled controls in the malathion and other studies. With this background in mind, Gross (1984) notes that there were but 15 rats in matched control groups as compared to 50 rats in each of the treatment groups. It is obvious that with so few matched control animals, statistical resolution is compromised with respect to that expected when GLP is followed. Furthermore, the 15 matched controls were divided into two groups of 10 and 5 rats each. These two subgroups of matched controls exhibited different weight gains, which served to further confound statistical treatment of the data.

Pooled controls were inherently poorly matched to animals under test. Gross (1984) indicates that pooled controls varied in number with time and were not strictly contemporaneous with exposed rats. It is apparent that, unlike matched controls, the pooled controls may not have been of the same strain and originating from the same supplier, and their tissues may not have been examined by the same pathologists as those who examined animals exposed to malathion or the matched controls. Furthermore, individual animal data for tumorigenic and non-neoplastic lesions are not presented for the pooled controls in the bioassay report. Without such information and in the face of so few matched controls, EPA cannot independently analyze the data.

There was no adjustment for tumor incidence on a life-table basis for proper analysis. As observed by Gross (1984), not only was this apparently not carried out by NCI, but the bioassay report contains no details that would enable anyone else (e.g., EPA scientists) to analyze in this way. (Note: An attempt was made by NCI to correct tumor incidence based upon the number of animals actually at risk, but was not an approach considered appropriate for an EPA assessment.)

The NCI study authors indicated that there were higher incidences of proliferative lesions of the thyroid gland in the dosed groups than in the matched control (page 24). However, the NCI statistical analysis of the findings for tumors concluded that there was no statistical evidence for carcinogenicity of malathion in male rats (page 28). In female rats combined follicular cell carcinomas/adenomas yielded a significant Cochran-Armitage test ($p = 0.026$) using pooled controls, but results of Fisher's Exact Test were not significant. (See Table 1). Thyroid tumors were not considered by NCI to be associated with administration of malathion (pages 37-38).

Gross (1984) performed independent statistical analyses of the NCI data and determined that for total tumors (adenomas and carcinomas of the thyroid there was a borderline positive Cochran-Armitage trend test for males ($p = .057$) and a highly significant trend test for females ($p = .018$). For this particular assessment, Fisher's Exact Test comparisons between controls and high dose groups were not statistically significant for either sex (Tox Branch calculation). Gross (1984) concluded that tumors of the thyroid gland were highly significantly increased in incidence (page 3). This reviewer is aware that many experts in the field of oncogenic assessment do not consider it appropriate to combine C-cell and follicular cell tumors of the thyroid. But such views do not alter these findings and the attendent concern that the thyroid gland may be a target for malathion. With respect to total proliferative changes of the thyroid (i.e., hyperplasia, adenomas, and carcinomas), there was according to Gross (1984) a highly significant trend for males ($p = .012$), borderline significance for females ($p = .075$) and a highly significant trend when the sexes were statistically combined ($p = .004$). Thus, according to Gross (1984), this strengthens the conclusion of carcinogenicity. This reviewer is also aware that experts in the field of oncogenicity generally do not agree that hyperplasia should be included with tumerogenic responses. However, it has been reported that C-cell tumors in humans appear to be preceded by a multifocal C-cell hyperplasia (Wolfe et al., 1973; as cited in Benirschke, et al., 1978, p. 493). Furthermore, Hill, et al. (1989) present information showing that follicular cell thyroid tumors may arise as a result of inhibition of thyroid-pituitary homeostasis. "The carcinogenic process proceeds through a number of stages, including follicular cell hypertrophy, hyperplasia and benign and sometimes malignant neoplasms" (page 629). Also, as will become evident in subsequent discussion, when NTP reexamined this study, many of the NCI diagnoses of hyperplasia apparently were identified as adenomas by NTP. Such uncertainties of characterization would suggest it appropriate in this case to consider combining proliferative lesions.

Computations by Gross (1984) reveal that when C-cell tumors alone are examined, neither the findings in males nor in females was highly significant. When follicular cell tumors alone were examined, while not significant for males, the finding among

females was highly significant for the trend (p = .027). Thus, in concurrence with NCI, Gross (1984) confirmed that in female rats combined carcinomas/adenomas of follicular cells yielded a significant trend, a finding shown in spite of the limited number of matched controls. Gross (1984) has labored to show that the same percentage findings in a normal size control group would have yielded a higher degree of significance as to the difference between dosed groups and controls and in terms of the trend test.

As alluded to above, NTP [Huff, et al. (1985)] reexamined this NCI study. The publication of this work concluded that the reexamination confirmed the original NCI interpretation that malathion was not carcinogenic. The extent to which the reexamination addressed the deficiencies identified in Gross (1984) with respect to the NCI study will become apparent.

Table 1 presents a comparison between those findings as reported by NCI and by NTP for the thyroid. NTP did not report incidences of hyperplasia, rather only adenomas and carcinomas. This table reveals remarkable disparities between the numbers of adenomas and carcinomas as reported by NCI and NTP pathologists. For example, for follicular cell adenomas among male animals, NCI reported incidences of 1, 1, and 1 for the matched control, low-, and high-dose groups, respectively, while NTP reported for the same respective groups 1, 7, and 8 adenomas. It appears from the comparison that many hyperplasias reported by NCI may have been identified as adenomas by NTP. Of course, lacking the individual NTP data, Tox Branch can only speculate that many hyperplasias as identified by NCI were viewed as adenomas by NTP. This is but one example illustrating the serious discrepancies between NCI and NTP pathologists, which scientists at EPA cannot resolve without the data. This reviewer has discussed the matter with Tox Branch pathologist, Dr. L. Slaughter, who has expressed the view that an independent reading of the study by EPA would be appropriate, provided all pertinent clinical, clinical pathology and other correlary pathology findings are provided the person(s) reading these malathion slides. Otherwise, EPA can only defer to NTP for a conclusion, which would violate the principle of independent review of studies by the Agency. In addition to discrepancies as to numbers of tumors, hyperplasia, etc., why were less animals reportedly examined by NTP, e.g., six fewer low-dose and seven fewer high-dose males were reportedly examined by NTP? Did NCI identify any tumors in those animals not counted and apparently not examined by NTP? Only an inspection of individual animal data will enable EPA to fulfill its mission of independent review and assessment. Another problem with the NTP reexamination is the appropriateness of examining selected tissues as opposed to a complete rereading of the entire pathology data base. The possiblility for changed or revised diagnoses should exist for all tissues in the study.

It should be noted that an additional significant deviation in this assay from EPA Guidelines was the limitation on dosing to 80 weeks followed by 29 to 33 weeks of observation. Guidelines require dosing throughout the 2-year study interval.

Tox Branch is of the opinion that the Osborne-Mendel rat study may reveal, albeit weakly, an oncogenic response for thyroid C-cells in male rats and thyroid follicular cells in male and female rats. At the very least, these findings support a reexamination by EPA pathologists and statisticians. These findings also support requiring the Registrant to include the oncogenic component in the required chronic rat study on malathion.

Table 1. Malathion - Osborne-Mendel Rats

Thyroid

		Male				Female			
		C^1	C^2	L	H	C^1	C^2	L	H
Number of Tissues Examined	1) NCI	14	46	41	47	15	46	48	49
	2) NTP	14	41	35	40	14	41	44	42
C-cell Hyperplasia*	1) NCI	0		1	3	0		5	3
	2) NTP	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C-cell Adenoma	1) NCI	0		1	3	0		1	2
	2) NTP	1		1	7	2		2	4
C-cell Carcinoma	1) NCI	0		0	0	0		0	0
	2) NTP	0		0	0	0		0	1
C-cell Adenoma and Carcinoma	1) NCI	0 (0%)	2 (4.3%)	1 (2.4%)	3 (6.4%)	0 (0%)	1 (2.2%)	1 (2.1%)	2 (4.1%)
	2) NTP	1 (7.1%)	3 (7.3%)	1 (2.9%)	7 (17.5%)	2 (14.3%)	10 (24.4%)	2 (4.5%)	5 (11.9%)
Follicular Cell Hyperplasia	1) NCI	1		7	8	0		3	0
	2) NTP	N/A		N/A	N/A	N/A		N/A	N/A
Follicular Cell Adenoma	1) NCI	1		1	1	0		0	1
	2) NTP	1		7	8	0		1	1
Follicular Cell Carcinoma	1) NCI	0		2	6	0		0	3
	2) NTP	1		2	4	0		0	3
Follicular Cell Adenoma and Carcinoma	1) NCI	1 (7.1%)	4 (8.7%)	3 (7.3%)	7 (14.9%)	0 (0%)	0 (0%)	0 (0%)	4 (8.2%)
	2) NTP	2 (14.3%)	8 (19.5%)	9 (25.7%)	12 (30.0%)	0 (0%)	1 (2.4%)	1 (2.3%)	4 (9.5%)

*Unable to locate hyperplasia for pooled controls in NCI report.
C^1 = Matched Controls
C^2 = Pooled Control
L = Low Dose
H = High Dose
N/A = Not available in publication

2. F344 Rat Study - Malathion

Doses of malathion employed in this study, 2000 and 4000 ppm, were less than one-half of those employed in the Osborne-Mendel rat study discussed previously. Equal numbers of animals were used in the matched control and dosed groups in this study. Even though dosage levels were less than in the case of the Osborne-Mendel rat study, mortality was high, especially in males. Survival at 103 weeks for males was 54, 28, and 0 percent for the control, low-, and high-dose groups, respectively. For females, the comparable figures were 64, 62, and 50 percent.

According to Gross (1984), animals in the high-dose group, particularly males, cannot be viewed as having been subject to the same risk as control animals to develop tumors appearing relatively late in their lifetime. Hence, proper assessment of tumorigenic response would require a life-table analysis. This apparently was not done by NCI, and data are not presented in the NCI study that would make it possible for anyone else (e.g., EPA reviewers) to perform the proper assessment.

Gross (1984) cites the following statement from the NCI study '"The incidence of adrenal (medullary) pheochromocytomas in the males, 2/49 (4%) in the control, 11/48 (23%) in the low dose animals and 6/49 (12%) in the high dose group, were not considered to be related to the administration of the test compound (malathion)"' (page 11). This statement was made in spite of the fact that the increased incidence at the low dose was statistically significant ($p = .006$) by Fisher's Exact Test, as acknowledged by NCI (page 22). The opinion rendered by NCI was predicated upon the lack of a significant effect at the high dose and lack of a dose response effect. However, Gross (1984) argues that the less remarkable observation at the high dose was ". . . without much doubt, due to poor survival of the males in that group . . . ." (page 11). Even without corrections for the high mortality, calculations by Gross (1984) show that a positive trend test ($p < .001$) and positive Fisher Exact comparisons ($p < .001$ for low dose; $p = .005$ for high dose) exist when low-dose and high-dose incidences of pheochromocytomas, 11/48(22.9%) and 6/49 (12.2%), respectively, are compared with the combined historical control plus contemporaneous control incidence of 10/324 (3.09%). It should be noted that pheochromocytoma was identified in two female rats in each of the dosed groups, but was not observed in the female matched control group. Gross (1984) concluded that pheochromocytoma was a positive finding in this study, which could be identified in spite of the lack of a life table analysis and in spite of possible competing toxicity (i.e., stomach inflammation and stomach ulceration in males).

The NTP reexamination of this study (Huff et al., 1985), affirmed the NCI assessment of no oncogenic finding. Neoplastic and certain non-neoplastic findings from the NCI study and the NTP reexamination of this F344 rat study are presented for purposes

of comparison in Table 2.

It is apparent in this comparison that increases in pheochromocytoma were not as remarkable by the NTP reexamination as by the NCI estimate. The differences in the two examinations rest in large part with the control groups, where for males the incidence reported by NCI was 4 percent and that by NTP was 10 percent. For females the respective incidences were 0 and 6 percent. Among male animals, this finding of three additional control animals and one less low-dose animal with pheochromocytoma alters the statistical significance. NTP advised that the low-dose group is not significantly different from control by the Fisher's Exact test. It should be noted that the historical control incidence of pheochromocytoma at this laboratory is reported as 3 percent among males (NCI, page 22), a figure more in line with the NCI estimates of the matched control incidence of 4 percent in this study than with the NTP estimate of 10 percent. Tox Branch is of the opinion that an independent reading of slides should be undertaken to resolve the discrepancies between the NCI and NTP assessment of pheochromocytoma and other pathologies in the study, or simply defer to the conclusions of NTP.

It is noteworthy that pheochromocytoma is often detected coincidentally in patients with medullary thyroid carcinoma. (Benirschke et al., 1978, p. 493).

Table 2 also reveals a remarkable disparity between NCI and NTP with respect to leukemia incidence. NCI did not comment upon leukemia incidence. NTP noted the increase at the low dose but dismissed it as a positive finding essentially due to the lack of a dose trend and lack of an effect at the high dose. Tox Branch can suggest here the same argument posed by Gross (1984) with respect to pheochromocytoma, namely that male rats in the high dose group were not as at risk to develop the neoplasm due to the high mortality in the group. In the NTP publication, the claim is made that survival-adjusted methods and Fisher's Exact/Cochran-Armitage tests were performed. However, with respect to pheochromocytoma and leukemia, the following statement is made: "There was also little evidence of a carcinogenic effect of malathion in male F344 rats, although the reduced survival in the dosed groups made the overall interpretation of these data somewhat more difficult. Life-table analyses (undefined) suggested that malathion may have increased the incidences of pheochromocytoma of the adrenal glands and leukemia in male F344 rats, primarily in the low-dose group. However, life-table analyses are appropriate only for those neoplasms clearly identified as causing deaths. In the malathion study the early deaths were due primarily to chemical toxicity, not the early onset of lethal tumors, and hence alternative methods of statistical analysis are preferable in this instance" (page 163). Findings were not significant by incidental tumor analysis or by Fisher's Exact test according to NTP. This is a very important point, since it would appear that a

positive effect was seen by life-table analysis, although the statistical results of this particular assessment were not provided beyond this statement. Thus, a principal concern in Gross (1984) with respect to the NCI study, no life-table analysis, was satisfied by NTP, and evidently demonstrated a positive finding but was then dismissed by NTP for both pheochromocytoma and leukemia. This reviewer is of the opinion that once selection of a method of statistical analysis is made, it is improper to then dismiss the findings because they might be troublesome or because, after the fact, one thinks the method of analysis was not germane. These findings at least cast doubt upon claims that this was a negative study and support the Registration Standard's call for expanding the required chronic F344 rat study to include the oncogenic component. Furthermore, slides from this study should be examined within the Agency, followed by the usual statistical analyses.

Table 2 also reveals certain remarkable non-oncogenic findings in the study. Stomach inflammation and ulceration were clearly increased in a dose-related fashion among males by both NCI and NTP estimates. Also, the NCI report identified increased incidences of fatty metamorphosis and focal cellular changes of the liver for females and chronic inflammatory change of the kidney in females. These latter findings in females were not reported in the NTP reexamination. These non-neoplastic findings further support the Agency's determination to require a full 2-year chronic toxicity study in the F344 rat. NCI noted (page 35) that gastric non-neoplastic lesions were found in F344 rats administered malathion and malaoxon, but were not detected in Osborne-Mendel rats administered malathion.

In conclusion, this reviewer agrees with Gross (1984) that an analysis of the NCI study indicates a positive oncogenic response, pheochromocytoma, identified without the much-needed life-table analysis. The NTP reexamination reports substantial shifts in pathologic diagnoses with respect to the NCI assessment and does not satisfy the concerns raised by Gross (1984) with respect to corrections for high mortality among male rats at the high dose. Neither NTP nor NCI provided in the cited documents the individual animal data that would be necessary to enable a second party such as EPA to perform statistical treatment of the data. The lack of this kind of presentation of information would be unacceptable in a contemporary study. It is therefore incumbent upon Tox Branch to examine the slides and obtain the necessary data to pursue the statistics after its own procedures.

The acceptability of this study is further weakened by the NCI assertion that the MTD may not have been reached in females (page 35).

Table 2. Malathion - F344 Rats

		Male			Female		
		C	L	H	C	L	H
Adrenal							
Pheochromocytoma	NCI	2/49(4%)	11/48(23%)	6/49(12%)	0/49(0%)	2/49(4%)	2/49 (4%)
	NTP	5/49(10%)	10/48(21%)	6/46(13%)	3/48(6%)	2/47(4%)	3/49 (6%)

"The historical control data for adrenal pheochromocytoma in untreated male F344 rats at this laboratory show an incidence of 8/275 (3%)." NCI, page 22.
Cochran-Armitage Trend Test: not significant per NCI
Fisher Exact Test: males, low dose (p = .006) per NCI

Stomach							
Inflammation, chronic	NCI	2/49(4%)	6/46(13%)	11/47(23%)	0/50(0%)	2/44(5%)	4/47(9%)
	NTP	0/48(0%)	5/48(10%)	7/48(15%)	0/48(0%)	2/46(4%)	2/47(4)
Ulceration	NCI	1/49(2%)	9/46(20%)	15/47(32%)	1/50(2%)	2/44(5%)	2/47(4%)
	NTP	2/48(4%)	8/48(17%)	17/48(35%)	2/48(9%)	4/46(9%)	3/47(6%)
Liver							
Fatty Metamorphosis	NCI	1/49(2%)	3/50(6%)	2/49(4%)	0/50(0%)	6/50(12%)	9/48(19%)
	NTP						
Focal Cellular Change	NCI	9/49(18%)	6/50(12%)	4/49(8%)	7/50(14%)	14/50(28%)	17/48(35%)
	NTP						
Kidney							
Chronic Inflammatory Change	NCI	36/48(75%)	42/50(84%)	42/49(86%)	16/50(32%)	30/49(61%)	31/49(63%)
	NTP						
Medullary Hyperplasia	NCI	4/49(8%)	3/48(6%)	0/47(0%)			
	NTP	11/49(22%)	12/48(24%)	4/49(8%)			
Leukemia	NCI	9/49(18%)	8/50(16%)	2/49(4%)	10/50(20%)	5/50(10%)	6/50(12%)
	NTP	13/50(26%)	20/50(40%)	8/49(16%)	10/50(20%)	9/50(18%)	9/50(18%)

III 83-1 and 83-2 Chronic/Oncogenicity - Rat - Malaoxon

The Registrant has requested an exemption from the requirement for a chronic/oncogenicity study in the rat using malaoxon as test material. The Registrant expresses the view that the oncogenic and chronic toxicity potential for malaoxon is essentially addressed by malathion oncogenicity and chronic toxicity testing since malathion is converted metabolically to malaoxon. Furthermore, the Registrant indicates that the Agency's rationale for requiring a study on a metabolite of malathion is not clear.

The Agency is requiring the study essentially for two reasons:

a. To provide clarification of the agent's oncogenic potential, and

b. To provide additional needed information on cholinesterase inhibition, since malaoxon is the active cholinesterase inhibiting entity.

The original NCI assessment of this study concluded that malaoxon was not carcinogenic in F344 rats.

Gross (1984) analyzed data from this study and found that for thyroid C-cell hyperplasia among male rats, where the respective control, low- and high-dose incidences were 0, 13.3, and 20.4 percent, there was a very highly significant Cochran-Armitage trend test ($p = < .001$) and positive Fisher's exact pairwise comparisons for both the low-dose ($p = .01$) and high-dose ($p = < .001$) groups. The NCI report did not provide statistical analysis of these C-cell hyperplasia data. Among female rats, the NCI assessment reported a positive finding for C-cell tumors (adenomas + carcinomas) as evidenced by a positive Cochran-Armitage trend ($p = .009$) and a positive ($p = .024$) Fisher's pairwise comparison for the high-dose group (NCI, page 22). The combined incidences of C-cell adenomas and carcinomas in question were 0, 2, and 11 percent for the control, low- and high-dose groups, respectively. These statistical findings were essentially confirmed by Gross (1984). In spite of this statistical assessment of the data, NCI dismissed the findings as evidence of oncogenicity based upon consideration of historical control data: "The historical record of this laboratory shows an incidence of female F344 rats with C-cell adenomas or carcinomas of 16/223 (7%), compared with 0/50 in the control group, 1/49 (2%) in the low-dose group and 5/47 (11%) in the high-dose group of this study. This indicates that the incidence of C-cell tumors of the thyroid in female rats of the present study is comparable to that usually seen in control animals" (page 23). This argument can hardly be used with respect to the subsequent NTP reeanalysis where control, low- and high-dose incidences were reported as 8, 15, and 23 percent (possibly 25%)(See Table 3). By NTP reanalysis, the incidences of C-cell adenomas and carcinomas for females were

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significantly increased at the high dose (p = .05) and, according to NTP, yielded evidence of a dose-trend. Furthermore, with NTP reanalysis there was a remarkable shift in reported incidence of C-cell hyperplasia among male animals which effectively washed out the highly significant finding for this parameter by NCI as discussed previously (see Table 3). However, the reanalysis revealed a postive finding, statistically, for adenomas and carcinomas among males of the high-dose group (p = < .05). Thus, with respect to malaoxon testing, the NTP reanalysis disclosed positive tumorigenic responses of thyroid C-cells for rats of both sexes. The concern is exacerbated by the finding of a total of six carcinomas among high-dose males and females as contrasted with only one carcinoma among the controls.

In view of these findings, NTP concluded that there was equivocal evidence of carcinogenicity for malaoxon in male and female F344 rats. NTP employs the term equivocal when in NTP's opinion there is evidence of a marginal increase of neoplasms.

This reviewer is concerned over the substantial differences in diagnosis of thyroid hyperplasia/neoplasia reported by NCI and NTP (Table 3). In any case, the data indicate a tumorigenic effect of malaoxon of the same tissue as possibly so affected by malathion in Osborne-Mendel rats, which must be resolved through the repeat performance of a chronic/oncogenicity study of malaoxon in the F344 rat by contemporary EPA Guidelines.

The NCI report revealed increases in the incidence of pheochromocytoma of the adrenal gland in male animals of both dosed groups relative to the contemporaneous control group: 3/47 (6.4%), 4/49 (8.2%), and 6/49 (12.2%) for control, low- and high-dose groups, respectively. These numerical increases for the dosed groups were not reported by NCI as statistically significant. However, Gross (1984) provided a statistical treatment of the data which compared the incidences of pheochromocytoma for the dosed groups with NCI historical control data, which included the contemporaneous control data from the malaoxon study and that from the malathion F344 rat study, where the combined historical control incidence was 13/377 (3.5%). The particular statistical comparison using this historical control revealed both a postive trend (p = .002) and a positive pairwise comparison for the high-dose group (p = .008).

The subsequent NTP reexamination resulted in a considerable revision in the incidences of pheochromocytoma: 5/50 (10%), 6/50 (12%), and 10/49 (20.4%) for control, low- and high-dose groups, respectively (Table 3). NTP did not indicate any statistically significant findings. The NTP data are suggestive of a trend that would be statistically significant if analyzed after the fashion of Gross (1984) using combined NCI historical control, malathion F344 rat control and malaoxon control data as described above. However, since NCI and NTP differ with respect to the

incidences of pheochromocytoma in control groups in both the malathion F344 rat and malaoxon F344 rat studies, it is likely the historical control incidence at NCI would also be different as interpreted by NTP. In lacking the latter NTP assessment of NCI's historical control incidence, Tox Branch has elected not to combine the data for purposes of analyzing the data as was done by Gross (1984). It nevertheless appears that the trend remains following the NTP reanalysis. It is particularly important to properly assess the pheochromocytoma data since the same pathologic finding was possibly present in the malathion F344 rat study. Furthermore, there is scientific evidence that pheochromocytoma occurs coincidentally in patients with medullary thyroid carcinoma. (Benirschke et al., 1978, p. 492).

It should be noted that by both the NCI assessment and the NTP reexamination, there was an increase in the incidence of mammary fibroadenoma/adenoma in the low-dose group, reported by NTP as statistically significant. NTP dismissed this finding as related to dosing since the effect was not observed at the high-dose and the incidence in the concurrent control was unusually low.

As was true in the case of the malathion F344 rat study, the NTP reexamination of the malaoxon study disclosed an increase in the incidence of forestomach ulcer.

At the very least, all of these findings support the requirement for a new chronic/oncogenicity study of malaoxon in the F344 rat by contemporary Guidelines, as set forth in the Registration Standard.

The Registrant did not comment on the additional needed information on the effects of malaoxon on cholinesterase data as explained in the Registration Standard. Toward obtaining more definitive data on cholinesterase inhibition, the Registrant should be advised to consult with Toxicology Branch as to the protocol for assessing cholinesterase inhibition prior to initiating the study.

Table 3. Malaoxon - F344 Rat

Thyroid

			Male			Female		
			C	L	H	C	L	H
Follicular Cell Adenoma	NCI	(P56M;P60F)	2/49(4%)	0/45(0%)	1/49(2%)	0/50(0%)	0/49(0%)	1/47(2%)
	NTP	(P167)	2/49(4%)	0/45(0%)	1/49(2%)	2/48(4%)	0/48(0%)	2/48(4%)
Follicular Cell Carcinoma	NCI	(P56M;P60F)	1/49(2%)	0/45(0%)	1/49(2%)	0/50(0%)	0/49(0%)	0/47(0%)
	NTP	(P167)	1/49(2%)	0/45(0%)	1/49(2%)	0/48(0%)	0/48(0%)	0/48(0%)
C-cell Adenoma	NCI	(P56M;P60F)	1/49(2%)	0/45(0%)	2/49(4%)	0/50(0%)	1/49(2%)	4/47(9%)
	NTP	(P167)	2/49(4%)	3/45(7%)	8/49(16%)**	4/48(8%)	7/48(15%)	8/48(17%)
C-cell Carcinoma	NCI	(P56M;P60F)	1/49(2%)	0/45(0%)	2/49(4%)	0/50(0%)	0/49(0%)	1/47(2%)
	NTP	(P167)	1/49(2%)	0/45(0%)	2/49(4%)	0/48(0%)	0/48(0%)	4/48(8%)
C-cell Hyperplasia	NCI	(P77M;81F)	0/49(0%)	6/45(13%)	10/49(20.4%)	6/50(12%)	8/49(16%)	6/47(13%)
	NTP	(P169)	8/49(16%)	11/45(24%)	8/49(16%)	24/48(50%)	24/48(50%)	25/48(52%)
C-Cell Adenoma and Carcinoma	NCI		2/49	0/45	4/49	0/50	1/49	5/47
	NTP	(P167)	3/49(6%)	3/45(7%)	10/49(20%)*	4/48(8%)	7/48(15%)	11/48(23%)*** stat?
Adrenal Pheochromocytomo	NCI		3/47(6.4%)	4/49(8.2%)	6/49(12.2%)			
	NTP		5/50(10%)	6/50(12%)	10/49(20.4%)			
Mammary Gland Adenoma	NCI					2/50(4%)	9/50(18%)	1/50(2%)
	NTP					2/50(4%)	9/50(18%)*	1/50(2%)

*p < .05 vs. control (NTP).
**p ≤ .05 vs. control (NTP) (Incidential tumor and Fisher's Exact Test).
*** may be [12/48(25%)]

IV 83-2 Oncogenicity - Mouse - Malathion

The Registrant disagrees with the requirement for an additional oncogenicity study in the mouse having decided that the 1978 NCI malathion study under discussion provides sufficient data to conclude that malathion is not oncogenic in the mouse.

The question of oncogenicity in this particular B6C3F1 mouse study centers upon the incidences in male mice of hepatocellular carcinoma and liver neoplastic nodules, where NCI concluded (page viii) that even though there was a dose-related trend ($p = .019$) and a positive pairwise comparison ($p = .031$) between the pooled and control and high-dose groups, there was "no clear evidence" of an association between malathion administration and tumor incidence. This opinion was justified by NCI in part by reliance upon $p = .025$ as the criterion of significance when Bonferroni adjustments are made. This opinion of NCI is essentially endorsed by the Registrant.

Additional points which the Registrant cites in support of his view that this is a negative study include the high dosage levels employed (8000 and 16,000 ppm), both of which exceed the OPP accepted upper limit dose of 1.0 g/kg/day in mouse oncogenicity studies; the high background incidence for this tumor; and published literature showing the high spontaneous incidence and variable rate of liver tumors in male mice. All of these points are here acknowledged.

Although the malathion B6C3F1 mouse study may not be considered to demonstrate, definitively, a tumorigenic response, Toxicology Branch has concluded that it cannot be accepted as a negative study, since the dose-related trend ($p = .019$) and the incidence at the high-dose ($p = .031$) were observed at levels the Agency normally considers significant ($p = .05$). Thus, because of questionable liver findings, another study is required. NTP did not reexamine this study.

Additional information emphasizing the questionable nature of the malathion mouse study is cited from Gross (1984) as follows (page 7):

a. Competing toxicity, particularly at the high dose, for both males and females may have inhibited the expression of tumor;

b. The small number of matched control animals (10/group);

c. Detailed information on neoplastic and non-neoplastic lesions of the pooled controls was not provided along with that for matched controls. Hence, independent evaluation of that data is obviated; and

d. Statistical analysis of hepatocellular carcinoma alone (males) (incidence: pooled control 5/49, low dose 7/48 and high dose 11/49) yielded a statistically significant Cochran-Armitage trend test (p = .048), yet reported as N.S.(not significant) in the NCI study [Table F1, page 93, as noted by Gross (1984)].

V. 83-2 Oncogenicity - Mouse - Malaoxon

The NCI study concluded that under the conditions of the bioassay, malaoxon was not carcinogenic in the B6C3F1 mouse. The Agency review of the study concurred with NCI in this opinion and, hence, the Malathion Registration Standard does not call for additional testing of malaoxon in the mouse. The Registrant is correct in reiterating the Agency position. Gross (1984) also acknowledges the study to be negative, but qualified that acknowledgement by noting a compromised, dose-related, availability of male animals at risk toward the end of the experiment and reduced female body weights in dose groups as evidence of possible competing toxicity.

VI. Cholinesterase Effects - Malathion

The following is a general discussion/assessment of available information on the cholinesterase inhibiting potential of malathion, the parameter upon which the tolerance and PADI for malathion are established.

Generally, the data base for cholinesterase inhibition resulting from long term exposures to malathion by ingestion is weak. The current RfD for malathion is based upon a human study (Moeller and Rider 1962) in which malathion was administered orally via gelatin capsules, to groups of five male subjects/dose group at doses of 8, 16, and 24, mg/day for 32, 47 and 56 days, respectively. Doses, as expressed on a 70 kg body weight basis, would be equivalent to 0.11, 0.23 and 0.34 mg/kg/day. The study reported no significant inhibition of RBC or plasma cholinesterases at the 8 and 16 mg/day doses, but reported significant inhibition of both enzymes at 24 mg/day. Hence, 16 mg/day has been used for regulatory purposes as a NOEL for cholinesterase inhibition.

The study in question is deficient in certain aspects. These include, but are not necessary limited to, the following. The authors claim that there was no "significant" inhibition of plasma or red cell cholinesterases at the two lowest doses, but that both enzymes were significantly inhibited at the 24 mg/kg/day dosage level. In making these claims, no calculations or statistical analyses are provided. The individual data are not

tabulated, but are reproduced only on crude graphs which plot cholinesterase activity versus time of exposure. It is evident that both enzymes were inhibited by about 25 percent at the high dose. It appears that neither enzyme was inhibited at 8 mg/day and it appears somewhat questionable as to whether plasma cholinesterase was inhibited at 16 mg/day. Maximum inhibition at 24 mg/day occurred for both enzymes some three weeks after cessation of administration of malathion (when tested at 24 mg/day, patients were administered malathion for 56 ~~weeks~~ days, but were monitored for an additional 78 days). It should be noted that at the lower doses, malathion was administered for less than 56 ~~weeks~~ days as indicated above, ~~and when discontinued at these lower doses, EPN administration~~ WHEREUPON MALATHION-EPN COADMINISTRATION was initiated. Hence, the testing protocol at the lower doses was not comparable to that at the highest dose. One would like to see equivalent periods of exposure and postexposure observation for all three dose groups. The individual data are not provided and the reader must accept the authors' claims of significance without independent confirmation. B.D. 2/8/9[illegible]

Source and purity of the test agent are not provided. The purity of malathion samples tested generally range from 65 to 99 percent. The reader must presume the compound was administered as a single dose once daily. It is emphasized that were but five male subjects/dose group. It is plain from the deficient reporting in the publication that the required independent assessment of this study by the Agency is impossible. It is quite feasible, for example, that inhibition of cholinesterase could have occurred at the lower doses and which by EPA statistical analysis could prove meaningful. Do the dosages employed, expressed in units of mg/day, refer to 99 percent malathion or a sample of lesser purity? The reader must presume that administered doses were properly corrected for impurities in achieving the level of malathion claimed to have been administered.

As reviewed in USEPA (1975) studies in the rat have shown the following:

a. Hazleton and Holland (1953)--Malathion (65%), as administered via the diet at 100, 1000, and 5000 ppm for 2 years, inhibited RBC, plasma and brain cholinesterases at 1000 and 5000 ppm, but not at 100 ppm. The Agency does not have the actual data to evaluate these claims;

b. Hazleton and Holland (1953)--Malathion (90%) as administered also via the diet at 100, 1000 and 5000 ppm for 2 years inhibited all three enzymes at all doses. The EPA review indicates that the depressions of the three cholinesterases were statistically significant at all doses (page 71). Actually, "slight" inhibitions were noted by the authors at the 100 ppm level, which the reader must conclude were statistically significant, if so inclined. In any

case, inhibition of all three enzymes at 100 ppm is indicated by the authors. The Agency does not have the actual data for evaluation; and

c. Golz and Shaffer (1956)--Malathion (99%) as administered to rats via the diet at 500, 1000, 5000, and 20,000 ppm for 2 years inhibited RBC cholinesterase at all dose levels. Hence, for this study, NOEL < 500 ppm. The actual data for this study are not available within Agency files.

Additional cholinesterase data for the rat are presented in the 1981 American Cyanamide 2-year study in the Sprague-Dawley rat performed by Food and Drug Research Laboratories discussed previously. Rats were administered malathion via the diet at 100, 1000, and 5000 ppm for 2 years. RBC cholinesterase was significantly inhibited at all three dosage levels. (Gross, 1989). Inhibition was dose-related over the entire 2-year duration of the study, cholinesterase activity (RBC and plasma) was measured at the 3, 6, 12, and 24-month time points. The magnitude of inhibition of RBC cholinesterase at 100 ppm averaged about 11 percent. Hence, this study did not identify a NOEL for RBC cholinesterase inhibition. Plasma cholinesterase was apparently not inhibited at 100 ppm in the study. Brain cholinesterase was not assayed.

It is the opinion of Toxicology Branch that these limited findings, taken as a whole, do not identify a definitive NOEL for cholinesterase inhibition in the rat resulting from a chronic exposure to malathion. Since the RfD/PADI for malathion is now based upon cholinesterase inhibition in a questionable human study, it is recommended that the rat and mouse oncogenic and chronic dog studies required in the Registration Standard be designed to provide definitive NOELs for RBC, plasma, and brain cholinesterases.

The Registrant is thus to be advised to consult with Toxicology Branch in order to best define the conduct of these studies, both malathion and malaoxon, in order to achieve definitive data on cholinesterase inhibition.

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