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

JUL 16 1990

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Identification No. 57875
Additional Information Concerning NCI's 1978 B6C3F1 Mouse Study, "Bioassay of Malathion for Possible Carcinogenicity"

Project No.: 0-1329
Tox. Chem. No.: 535
Record No.: 265011

TO: Joanne Edwards, Review Manager
PM Team #74
Special Review
and Reregistration Division (H5708C)

FROM: Brian Dementi, Ph.D., D.A.B.T.
Review Section I
Toxicology Branch I
Health Effects Division (H7509C)

Brian Dementi 7/12/90

THRU: Roger Gardner, Acting Section Head
Review Section I
Toxicology Branch I
Health Effects Division (H7509C)

Karl Gardner for 7/14/90

CONCLUSION

An evaluation of data submitted by American Cyanamid Company on May 7, 1990 concerning the National Cancer Institute's 1978 carcinogenicity bioassay of malathion in the B6C3F1 mouse (NCI Technical Report Series No. 24) does not serve to alter the requirement for a new malathion mouse carcinogenicity study as set forth in the February 1988 Malathion Registration Standard.

COMMENTS

In the NCI study, malathion was administered to groups of 50 mice of each sex at one of two doses, either 8,000 or 16,000 ppm, for 80 weeks, and then observed for 14 or 15 weeks. Matched controls consisted of groups of 10 untreated mice of each sex. Pooled controls consisted of the matched controls combined with 40 untreated male and 40 untreated female mice from similar

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bioassays of four other test chemicals. All surviving mice were killed at 94 or 95 weeks.

The tumorigenic response of concern in this study was that of the liver in male mice. The NCI study presented the following tumor incidences (combined neoplastic nodules and hepatocellular carcinoma):

Matched Control (%)	Pooled Control (%)	8000 ppm (%)	16,000 ppm (%)
2/10 (20)	8/49(16)	7/48 (15)	17/49 (35)

According to NCI's calculations the Cochran-Armitage trend test yielded P=.041 when the matched control was used and P=.019 when the pooled control was used. Fisher's exact comparisons between the high dose group tumorigenic incidences and that of the pooled control yielded P=0.031. Findings for the other dosed group-control group comparisons were not significant at $P \leq .05$.

The February 1988 malathion Registration Standard concluded, based upon the Toxicology Branch review of the study, that because of study design flaws and the liver findings (i.e., dose-related trend, P=0.019, and increased incidence of liver tumors at the high dose, P=0.031), another study in mice is required. (P.17)

In an effort to establish that the study is actually an acceptable negative study which should satisfy the Section 83-2 Guideline requirement for carcinogenicity testing in the mouse, American Cyanamid Company has submitted additional information on the study via letter of R. L. Linkfield, Chairman, Technical Committee, Malathion Reregistration Task Force to Ms. Lois Rossi, Office of Pesticide Programs, dated May 7, 1990.

In re-analyzing the NCI data, American Cyanamid excluded from consideration those animals that died on test prior to week 58, the time point at which the first liver tumor incidence of the study occurred. Thus, the total number of male mice at risk by this interpretation were 9 in the matched control (as opposed to 10 by NCI) and 47 (48 by NCI) and 49(49 by NCI) in the 8,000 and 16,000 ppm dose groups, respectively:

Matched CONTROL (%)	8,000 PPM (%)	16,000 ppm (%)
2/9 (22)	7/47 (15)	17/49 (35)

As conducted by American Cyanamid and confirmed by the Toxicology Branch statistical team analysis (Hugh Pettigrew), Fisher's exact tests for each of the two dosed groups vs. the matched control was not significant at $P \leq .05$. American Cyanamid

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acknowledges significant Cochran-Armitage trends for neoplastic nodules and for combined neoplastic nodules and hepatocellular carcinoma at $P \leq .05$, but indicates that "the single statistical test in the absence of a positive Fisher's exact test does not establish a dose response." Toxicology Branch elects to offer no comment at this time with respect to an interpretation of what "establishes" a dose-response.

It should be recognized that neither NCI nor Toxicology Branch reported a positive Fisher's exact test for either dosed group in comparison to the matched control using the original NCI incidence data. The positive Fisher's exact test (P=0.31) resulted from the comparison between the high dose group incidence and the pooled control incidence. Since the high dose group combined tumor incidence of 17/49 is unchanged by American Cyanamid's analysis and since American Cyanamid's submittal did not include pooled control data, Toxicology Branch must conclude that the original assessment is unaltered with respect to this particular statistical aspect to the study.

Furthermore, it should be noted that Cochran-Armitage trend test results have slightly lower P values as a consequence of the American cyanamid adjustment. Using matched controls, the original NCI trend test result of P=0.041 becomes P=0.034, and using pooled controls, the original trend test result of P=0.019 becomes P=0.013 after the adjustment. Thus, the tumor incidence alterations slightly enhance the significance of the already positive dose trends and do not influence the Fisher's exact comparisons involving the pooled control group, which was positive (P=0.031) for the high dose group comparison.

Toxicology Branch recognizes that the matched control group is the preferred group to be used in statistical and biological assessments, however, in the study, the small size of the matched control group, 10 animals, shifts much of the burden upon the pooled control group for use in determining whether there has been a test agent related effect.

Hence, the exclusion of one additional animal from the matched control and low dose groups as not having been at risk does not alter the requirement set forth in the Registration Standard for another malathion study in the mouse.

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

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MEMORANDUM

SUBJECT: EPA Reg.241-110; 241-208, Malathion; NCI Cancer Bioassays of Malathion and Malaoxon. CASWELL=535; Accession:242903

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

FROM: William Dykstra, Toxicologist
Toxicology Branch, HED (TS-769)

TO: William Miller (15)
Registration Division (TS-767)

Recommendations:

1) Malathion was not considered carcinogenic to Osborne-Mendel rats, F344 rats or female B6C3F1 mice in the studies reported. Because of questionable liver findings in the male mice, another study in mice is required.

2) Malaoxon was not considered carcinogenic to F344 rats B6C3F1 mice in the study reported.

Review:

1) Bioassay of Malathion for Possible Carcinogenicity (NCI Carcinogenesis Technical Report Series No. 24, 1978; CAS#121-75-5; NCI-CG-TR-24)

A bioassay of technical-grade malathion for possible carcinogenicity was conducted by administering the test chemical in feed to Osborne-Mendel rats and B6C3F1 mice. Groups of 50 rats of each sex were administered malathion at one of two doses for 80 weeks, then observed for 33 weeks. Time-weighed average doses were 4,700 and 8,150 ppm. Matched controls consisted of groups of 15 untreated rats of each sex; pooled controls consisted of the matched controls combined with 40 untreated male and 40 untreated female rats from similar bioassays of four other test chemicals. All surviving rats were killed at 108-113 weeks.

Groups of 50 mice of each sex were administered malathion at one of two doses, either 8,000 or 16,000 ppm, for 80 weeks, then observed for 14 or 15 weeks. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls consisted of the matched controls combined with 40 untreated male and 40 untreated female mice from similar bioassays of four other test chemicals. All surviving mice were killed at 94 or 95 weeks.

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Results:

Mortality in either rats or mice was not significantly related to the administration of malathion. Sufficient numbers of animals were at risk in the dosed and control groups of rats and mice of each sex for development of late-appearing tumors.

In female rats, three follicular-cell carcinomas and one follicular-cell adenoma of the thyroid occurred in the high-dose group, and three follicular-cell hyperplasias occurred in the low-dose group.

The incidence of three tumors showed a statistically significant (P = 0.026) dose-related trend; however, the results of the Fischer exact test for direct comparison between the dosed and control groups were not significant. More dosed males than females had either tumors or hyperplasia of the follicular cells of the thyroid; however, because of the higher incidence of tumors among the male controls, none of the results of the statistical tests were significant. These thyroid tumors were not considered to be associated with the administration of malathion.

In male mice, hepatocellular carcinoma occurred at the following incidences: matched controls 2/10, pooled controls 5/49, low-dose 7/48, high-dose 11/49. In addition, neoplastic nodules occurred in 3/49 pooled-controls and 6/49 high-dose animals. When the combined incidence of these neoplasms in the dosed animals was compared with that of the pooled controls, the dose-related trend was P = 0.019 and the direct comparison of the high-dose group with the control group was P = 0.031. Thus, when NCI compared this high dose group with the control group using Bonferrone criteria, the difference was not significant since a P of 0.025 was required. Although NCI did not consider these liver tumors to be associated with the administration of malathion, the Agency has concerns about acceptability of this study as a negative study to fulfill our registration requirement for a mouse oncogenic study. Since there was a dose-related trend (P = 0.019) and an increase of tumors at the high dose (P = .031) at levels which the Agency normally considers to be significant, we believe that there is sufficient justification to require another mouse oncogenic study.

Conclusion:

Under the conditions of this bioassay, there was no clear evidence of the association of the tumor incidence with the administration of malathion to Osborne-Mendel rats or B6C3F1 mice. However the questionable increases of liver tumors limit its usefulness as a negative oncogenic study to fulfill a regulatory requirement.

Classification: Core-Minimum Data

United States
Environmental Protection
Agency

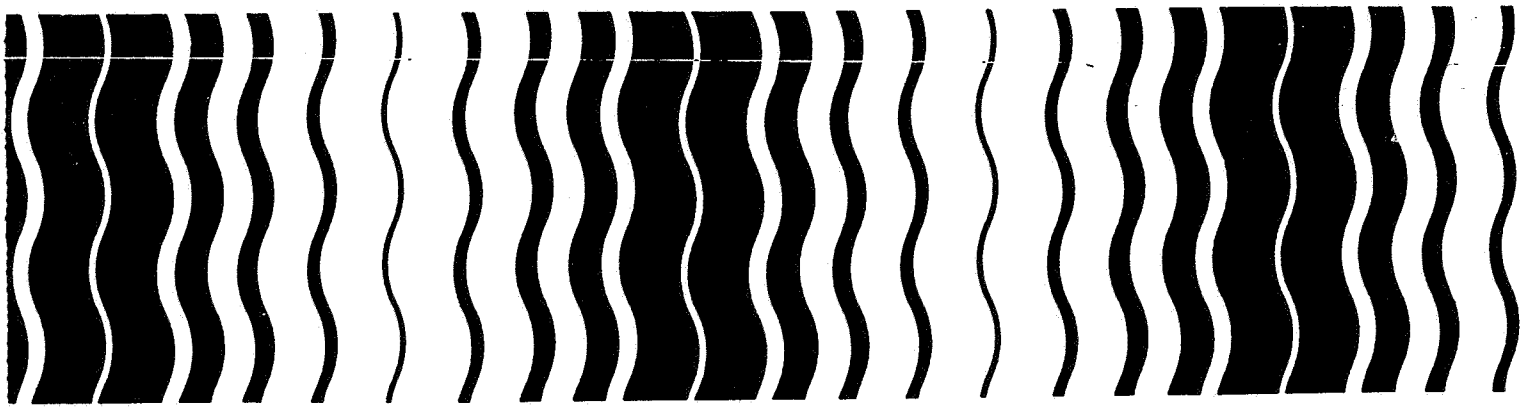
Office of
Pesticides and Toxic Substances
Washington DC 20460

FEBRUARY 1988

Pesticides

EPA

Guidance for the Reregistration of Pesticide Products Containing Malathion as the Active Ingredient



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acceptability of this study. These must include all doses and both sexes for the following tissues: liver, lung, kidney, pituitary, spleen, lymph nodes, mammary gland, reproductive organs, adrenal gland, skin/ear, pancreas, thyroid, and heart. The reevealuation must include a grading of the severity of identified changes. The slides must be reread blind, without prior knowledge of dose level.

d. NCI/NTP Malathion Oncogenicity Study in the B6C3F1 Mouse

In this study, malathion (purity 95%) was administered to B6C3F1 mice via the diet at one of two doses, either 8000 or 16,000 ppm; for 80 weeks. It was then removed from test diets and the mice were observed for 14 to 15 weeks.

In evaluating this bioassay, NCI/NTP concluded that under the conditions of the study, there was "no clear evidence" of an association between malathion administration and tumor incidence. The report noted a possible increased incidence of hepatocellular carcinoma in male mice. This tumor was observed at the following incidences: matched controls 2/10, pooled controls 5/49, low dose 7/48, and high dose 11/49. Neoplastic nodules occurred in 3/49 pooled controls, and 6/49 high-dose animals yielding overall incidences of 8/49 in pooled controls and 17/49 in the high dose group. When combined, using pooled control data, there was a dose-related trend ($P = 0.019$). For the direct comparison of the high-dose and pooled control groups ($p = 0.031$ Fisher's Exact Test), NCI/NTP employed as its criterion of significance $p = 0.025$, based on Bonferroni adjustments, and hence did not consider this a positive finding. A similar conclusion was reached by NCI/NTP with respect to data derived by time-adjusted analyses: matched controls, 2/9; pooled control, 8/48; low dose, 7/47; high dose, 17/49. NCI/NTP noted that historical control incidence data for hepatocellular carcinoma in this strain of mouse often is higher than that observed in the high-dose group seen in this particular study. This study was not reexamined by the NCI/NTP.

Because of study design flaws and the questionable liver findings (i.e. dose-related trend ($p = 0.019$) and increased incidence at hepatocellular carcinomas at the high dose ($p = 0.031$), another study in mice is required.

5. Oncogenicity (Metabolite)

The NCI/NTP has performed oncogenicity studies on the cholinesterase-inhibiting malathion metabolite malaoxon, in the F344 rat and the B6C3F1 mouse. These studies are discussed below.

P. 17 from MALATHION Registration Standard