Quaife 3-15-77

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE: MAR 15 1977

SUBJECT: Oxamyl, substantive amendment to PP No. 6F1696 of 10/29/76, TB evaluation of.

001718

FROM: TB/RD

TO: Mr. Frank Sanders

PP No. 6F1696

E. I. du Pont de Nemours & Co.
Wilmington, Delaware 19898

CONCLUSIONS:

1. Deficiency 2 of EPA 2/13/76 reject letter, this PP, is not satisfied. From the 90-day rat feeding study on DMCF (Sec. C, Exh. 3, this submission), Petitioner is asked to submit (detailed) microscopic findings on eyes and kidneys of all (including 50- and 150-ppm) rats not previously studied, with respect to:

   a, Hydronephrosis (Kidney)
   b. Minute foci of mineralization (kidney)
   c. Localized focus of retinal atrophy (eye)

2. Deficiency 3 of letter is satisfied (cf. TB 11/18/76 memo, PP No. 6F1695).

3. Deficiency 4 of letter is not satisfied for lack of mouse or hamster oncogenicity study.

4. Deficiency 5 of letter is partially satisfied; however, further mutagenicity data are likely to be needed, depending on content of "Sec. 3" guidelines, when revised.

5. Deficiency 6 of letter is not completely satisfied. We await Petitioner's reply to questions a, b, and c in Conclusion 3 of TB 11/18/76 memo, PP No. 6F1965, before commenting further on this. In addition, Petitioner is asked to explain how (as claimed in Exhibit 1, 6/8/76 submission, PP No. 6F1965, pp. 10 and 11 and Figs. 5 and 6) the rat could have synthesized the C^{14}-containing "essential amino acids," leucine, tryptophan, lysine, methionine, and phenyl alanine, from fed C^{14}-labelled oxamyl, when the rat is presumed to be incapable of synthesizing the essential amino acids.

RECOMMENDATION:

TB is unable to recommend for the requested tolerance, this PP, until deficiencies noted in "Conclusions" 1, 3, 4, and 5, above, are satisfied.

INTRODUCTION:

Petitioner's amendment responds to EPA reject letter of 2/13/76, this PP. A tolerance on tomatoes is requested.

New TOX data are supplied in 11 Exhibits in Sec. C of this amendment. They are reviewed in the attached appendix. (9 pp).

Preceding that is a "Discussion" of significant findings in Petitioner's present submission.

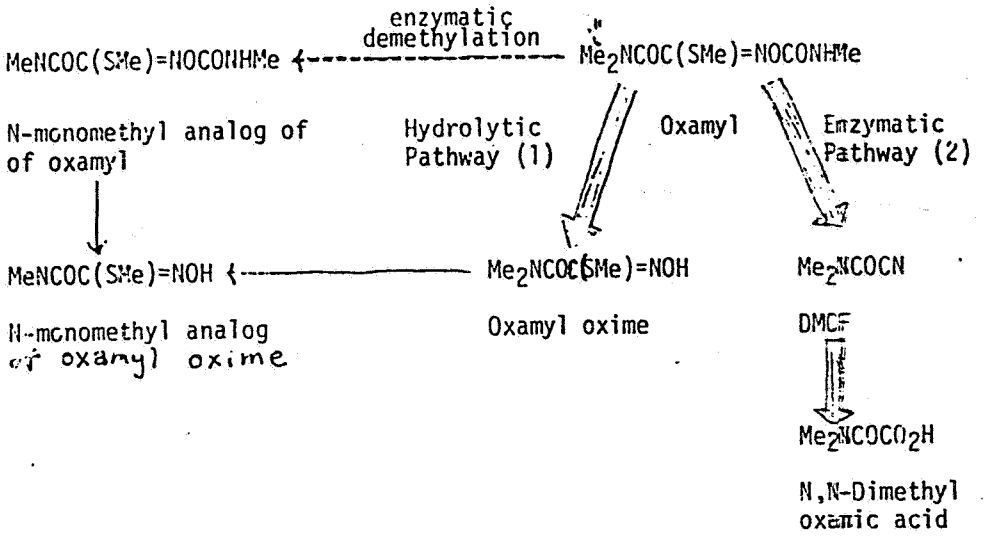
DISCUSSION:

New information on mammalian (rat) metabolism of oxamyl and TB evaluation of it.

Petitioner's findings and conclusions given immediately following are judged valid.

Liver microsomes degrade oxamyl by (1) hydrolysis to oxamyl oxime, which is resistant to further degradation and (2) enzymatic conversion of oxamyl to DMCF, then to N,N-dimethyl oxamic acid. At the same time, enzymatic demethylation processes convert the N-dimethyl carbamoyl group to the N-monomethyl carbamoyl group. See Fig. 1 on next page.

Fig. 1. Liver microsomal metabolism of oxamyl (rats)



Conjugates of oxamyl oxime and its N-monomethyl analog are thus end-products of pathway (1), including N-demethylation.

Conjugates of monomethyl and dimethyl oxamic acids are end-products of pathway (2), including N-demethylation (Exhibit 6, Sec. C, this PP, p.7).

Therefore, Petitioner contends, rats treated with oxamyl must convert "a large portion of oxamyl to DMCF and then to oxamic acids, which are eliminated as conjugates."

Indeed, conjugates of these four (oxamyl oxime, dimethyl oxamic acid, and the N-monomethyl analog of each) comprise ca. three-fourths of the radioactivity in urine and feces of rats fed C^{14}-labelled oxamyl (Exhibit 1, 6/8/76 amendment to PP No. 6F1965).

And a rat fed C^{14}-labelled DMCF eliminated 22% of radioactivity in urine as conjugates of the oxamic acids; they were the only C^{14} labelled compounds identified (Exhibit 5, Sec. C, this PP).

Both above findings are consistent with the pathway shown in Fig. 1.

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Conversely, 30% of Metabolite A (monglucose conjugate of oxamyl oxime), fed to a rat, appeared unchanged in the urine. This coincides with its metabolic inertness to rat liver in vitro (exhibit 6, Sec. C, this PP).

However, conjugates of these same four oxamyl-derived substances occurred in rat urine after Metabolite A was fed (Exh. 3, PP No. 6F1965, 6/8/76). So at least part of it is broken down in the living animal.

We conclude that if Metabolite A is degraded, then oxamyl oxime may have been degraded in the living animal. Glucose may be split off from Metabolite A in the G.I. tract, but where the oxamyl oxime moiety might be metabolized further is not known; liver microsomes did not degrade oxamyl oxime in vitro (Exh. 6, Sec. C, this PP).

As noted, the pathway shown in Fig 1 on preceding page, is judged valid in explaining, partially, the metabolism of oxamyl fed to rats.

However, we do not find that the evidence provided in Exhibit 5, this PP, proves Petitioner's claim that a significant portion of C^{14} from C^{14}-oxamyl fed to rats is first contained in DMCF and then incorporated into amino acids of blood, tissues, and urine.

The "evidence" of Exh. 5 (on incorporation of C^{14} in amino acids) consists of chromatographic behavior of solubilized radio-active substances from (blood, tissues, and urine of) a rat fed C^{14}-DMCF and some resulting radioactive bands or spots which gave a color with ninhydrin reagent.

We are unable to deduce (and Petitioner does not suggest) what fragment of DMCF would be incorporated into amino acids; by what known biochemical routes incorporation might proceed; or which amino acid(s) might be produced.

Petitioner does give evidence that the oxamic acids are not tied up with the "amino acid fraction."

Previously, Petitioner did seem to provide evidence for in vivo conversion of oxamyl to naturally-occurring amino acids. GLC separation of derivatized C^{14}-amino acids from acid-hydrolyzed skin/hair (or blood) of a C^{14}-oxamyl-dosed rat was carried out. The chromatogram "peaks" are labelled as specific amino acids (PP No. 6F1965, amendment of 6/8/76, Exhibit 1, "Metabolism of Oxamyl in the Rat," by J. G-Y. Han et al., pp. 10-11 and Figs. 5 and 6).

However, doubt is cast on validity of this finding; because these labelled amino acids include several which are "essential" to the rat and which the rat cannot synthesize (White, Handler, and Smith, "Principles of Biochemistry," McGraw-Hill, N.Y., 4th ed., p. 540) - leucine, tryptophan, lysine, methionine, and phenyl alanine.

It is, however, other information on rat metabolism of oxamyl that we have sought, i.e. (1) whether oxamyl and/or its metabolites conjugate in vivo with, e.g., protein or nucleic acids and (2), if so, whether such conjugates could plausibly exert physiologic/pathologic effect in vivo. [Because the oxamyl oximes form glucose and polyglucose conjugates in plant metabolism, they might logically be expected to form conjugates in mammalian metabolism.]

Such information is sought, especially because of the relatively large amounts of such glucose conjugates in some oxamyl plant residues (for which the proposed enforcement method does not analyze).

To date, however, Petitioner has not satisfactorily answered our concern in this regard (noted in TB memos of 1/6/75, this PP, and 11/18/76, PP No. 6F1695).

Nex TOX data.

Mutagenicity of oxamyl was tested for effects in bacteria in "recassay;" "reverse mutation" (Ames) test, with and without added rat liver metabolizing system; and host-mediated assay, in vivo and in vitro. All results were negative.

In 90-day rat feeding of DMCF, an oxamyl metabolite, a "no-effect level" could not be determined for lack of microscopic study of intermediate-dose-group rats - for eye and kidney effects seen in top-dose rats. Other toxic effects, including reduced weight gain and depressed RBC and hemoglobin, occurred in top and middle-dose rats. A one-litter reproduction test conducted on a sub-group showed 150 ppm DMCF to be "no-effect;" weanlings from 450-ppm rat parents showed markedly lower body weights.

In metabolism study of C^{14}-labelled oxamyl in the tomato, Petitioner found DMCF present in ripe fruit (4%), along with Metabolite A (5%); free oxamyl (59%); oxamyl oxime (13%); and polar metabolites (19%).

M.L.Q., 3/9/77
M.L. Quaife, Ph.D.
TB/RD
Ed. cer 3/15/77

Exhibits of Sec. C., PP No. 6F1696.

Exhibit 1. "Livestock Feeding Study"

Milk and tissue samples from the study, "Oxamyl Livestock Feeding Studies, Milk and Meat," du Pont Co., January, 1973 (EPA, PP No. 3G1316), were analyzed for the oxamyl metabolite, DMCF. No detectable residues (<0.02 and 0.04 ppm, respectively) were found. Cows had received 20 ppm oxamyl in the diet. A gas-liquid chromatographic analytical method for DMCF is included. (However, we noted no data to show that such old samples would be stable with respect to content of DMCF.)

Exhibit 2. Oxamyl Metagenicity Studies Using Bacteria.

Oxamyl, 93.7% pure, was tested for mutagenicity in four systems, as detailed below. It was found negative in all of them.

1. Recassay - Y. Shirasu et al., Mutation Res. 40, 19-30 (1976).

   Method. "DNA damage capability" was checked with B subtilis H-17 (rec.+) and M-45 (rec.-). Both bacterial strains were streaked on an agar plate. A small paper disk soaked in a solution of oxamyl was placed so as to cover an end section of each streak. After incubation, the length of each streak which showed growth inhibition was measured. Mutagens cause different degrees of inhibition. Non-specific inhibitors (e. g., certain antibiotics) cause the same amount of inhibition. Non-mutagens do not inhibit.

   Results. No inhibition was observed on oxamyl-treated bacterial streaks on agar plates incubated at 37° C. Mitomycin C, positive control, showed marked difference in degree of inhibition between the two strains. Kanamycin, negative control, caused equivalent inhibition in the two strains.

   Conclusion. Results are negative for mutagenicity of oxamyl.

2. Reverse mutation test - Ames et al., Proc. Natl. Acad. Sci. USA 72, 979-83 (1975); 70, 2,281-5 (1973).

Method. Ames' strains of S. typhimurium, TA 1535, TA 1536, TA 1538, TA 98, and TA 100 (histidine auxotrophs) and E. coli WP2 hcr^{-6} (which requires tryptophan for growth) were used. Each was mixed with agar and either histidine-biotin or tryptophan. Each mixture was plated with oxamyl, with or without the addition of a drug-metabolizing system (containing the 9,000 x g liver supernatant of a rat treated with polychlorinated biphenyl). After 2 days' incubation at 37° C., numbers of revertant colonies were counted. As positive control chemicals, 2-aminoanthracene, AF-2 [2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide], beta-propiolactone, 9-aminoacridine, and 2-nitrofluorene were used.

Results. The liver supernatant activated 2-aminoanthracene; numbers of revertant colonies of the TA strains were distinctly increased, compared to the control. The other four positive-control chemicals, without metabolic activation, also increased numbers of revertant colonies. However, oxamyl, with or without liver supernatant present, did not cause such increase with any strain of bacteria.

Conclusion. Oxamyl was negative for mutagenicity in this test. And to the extent that any oxamyl metabolites may have been formed by the liver supernatant in the test, they too were negative.

3. Host mediated assay in vitro.

An in vitro reverse mutation test using the bacterial strain, S. typhimurium G 46 hys, was conducted. Numbers of mutation colonies per plate were found not to be affected by presence of oxamyl; although beta-propiolactone (positive control) caused an increase.

Conclusion. Oxamyl was negative for mutagenicity in this test.

4. Host mediated assay - Legator and Malling, "Chemical Mutagens: Principles and Methods for their Detection," Ed., Hollaender, Plenum Press, N.Y., v.2, pp. 569-89 (1971).

Method. Groups of 5 or 6 male mice each received by stomach tube either water, 2 or 4 mg/kg oxamyl, or 50 mg/kg dimethylnitrosamine (positive control). All mice then received ip a suspension of S. typhimurium G 46 hys.

After 3 hours, mice were killed; phosphate buffer was injected into each mouse ip; aliquots of peritoneal fluid were removed and added to agar (not containing biotin); and these were plated and incubated at 37° C. Revertants and survivors were then counted.

Results. Oxamyl did not increase the number of revertant colonies significantly above the control; while the positive control did ($p<0.01$).

Conclusion. Oxamyl was not mutagenic in this assay.

Exhibit 3. Ninety-day feeding study in rats with 1-cyano-N,N-dimethyl-formamide (INN-79), metabolite of oxamyl (Vydate^R). Haskell Lab. Rept. No. 630-76, 8/26/76.

Introduction. DMCF, 1-cyano-N,N-dimethylformamide, is a possible cyanogenetic metabolite of oxamyl. Its approximate lethal dose is 450 mg/kg body weight for male rats. Given at repeated doses of 90 mg/kg/day for a total of 10 doses over two weeks, it caused significantly decreased ($p<0.05$) BW and rate of weight gain and significantly decreased ($p<0.05$) absolute weights of liver and kidney and absolute and relative weights of spleen and thymus; the absolute weight of the testes was decreased, but the relative weight was increased compared to controls. Three rats dosed with the test compound and killed after the tenth dose showed slight thymus, bone marrow, and spleen atrophy. This change was not seen in rats killed after 14 days' recovery. Acute pancreatitis was seen in one rat killed after the tenth dose, and loss of cytoplasmic vacuolation of hepatocytes in the centrilobular area of the liver was seen in all treated animals, with partial recovery of the latter after 14 days. (These findings are described in the title report (above); no raw data are given.)

Ninety-day feeding study.

Method. Groups of 16 M and 16 F ChR-CD rats, ca. 100 g in weight, each received 0, 50, 150, or 450 ppm 1-cyano-N,N-dimethylformamide (DMCF), 100% pure, Haskell No. 10,058, in the diet for 90 days. A 10% increase in test compound was added to the diet to compensate for evaporation during 24 hours at room temperature (confirmed by chemical analysis of diets). Observations made include body weight (weekly), food consumption, and behavior (daily). After ca. 30, 60, and 90 days, hematologic, urinalysis (on 24-hour samples), and blood chemical studies were made on 10 males and 10 females

of control, 150-ppm, and 450-ppm groups; if effect was seen, then 50-ppm rats were tested. After 90 days, ten surviving rats from each sex/dose-group were killed and examined grossly, and selected organs were weighed. Tissues from control and 450-ppm rats were examined microscopically. Some tissues were to be analyzed for content of DMCF.

(Following were examined or tested for: Hematology - RBC, hemoglobin, hematocrit, and WBC, total and differential. Urinalysis - volume, concentration (milliosmols/liter), sugar, protein, occult blood, pH, color, appearance, and microscopic appearance of sediment. Blood - alkaline phosphatase (AP), glutamic-pyruvic (GPT) and glutamic-oxalacetic (GOT) transaminase, & lactic dehydrogenase (LDH) activities, and total protein. Organ weights - brain, heart, lung, liver, spleen, kidneys, testis, stomach, adrenal, and pituitary. Tissues for microscopic exam. - trachea, lung, heart, aorta, esophagus, stomach, small intestine, cecum, colon, salivary gland, liver, pancreas, thyroid, parathyroid, adrenal, pituitary, kidney, urinary bladder, testis, epididymis, ovary, uterus, prostate, spleen, thymus, lymph nodes, bone, bone marrow, brain, peripheral nerve, eye, skin, mammary gland, and skeletal muscle. Some tissues samples were sent for residue analysis: brain, liver, kidney, spleen, muscle, testis, fat, cardiac blood, urine, and feces.)

Results. All rats survived the test. Rats on 450 ppm gained significantly less body weight; females at 150 ppm also gained less, but not significantly. Food efficiency was not affected. "Sporadic incidences of lacerations in shoulder and head regions," seen in controls and rats from all test groups, are attributed to hyperactivity. Two female rats at 450 ppm had exophthalmus and alopecia. One female rat at 50 ppm had loss of balance and head turned to one side - not attributed to DMCF. Male and female rats at 450 ppm had significantly reduced RBC, hematocrit, and hemoblobin; females had lower WBC's, as well. Similar changes were seen in 150-ppm rats, but only hematocrits and RBC's of males and RBC's and WBC's of females differed significantly. Female 450-ppm rats had lower urine osmolality - significantly so at 2 months.

Rats at 50 ppm were examined for hematologic, urinalysis, and clinical chemical values only at 3 months. Whether statistical tests for significant differences were made is not clear. Results listed do not appear to show any profound differences from control values.

No gross lesions were found in rats examined which are ascribed to treatment. No microscopic findings are judged related to treatment by the investigators. However, kidney findings are unusual: One/10 male and 2/10 female controls had hydronephrosis, but 7/10 male and 2/9 female rats at 450 ppm showed it. None/10 male and 3/10 female controls and none/10 male and 9/9 female rats at 450 ppm showed minute foci of mineralization (in kidney). In eyes, one of 20 controls and 5 of 19 rats at 450 ppm had localized focus of retinal atrophy.

Conclusion: A clear no-effect level is not shown. Microscopic examination of kidneys and eyes of all remaining rats is needed to show whether the incidences of the respective effects, noted microscopically for 450 ppm-rats, are dose- and compound-related; this need is confirmed by E. Long, M.D., Pathologist at EPA (told to Dr. M. Quaife, 2/10/77).

Reproduction Study.

Method. Six M and 6 F rats each from controls and from each group that had been fed dietary DMCF for 90 days were mated - in a one-generation, one-litter reproduction test. Records were kept of all matings; number of pregnancies; number of young, live and dead, in each litter at birth and at 4, 12, and 21 days of age; and body weights of weanlings (at 21 days). Litters with more than 10 pups were reduced to 10 at day 4. Following indices are given: Fertility (% pregnancies of matings), gestation (% live litters of pregnancies), viability (% rats alive at day 4 of rats born), and lactation (% rats alive at day 21 of rats alive at day 4).

Results. Weanling weights of 450-ppm pups are significantly (ca. 1/4) lower. Rats at 150 ppm showed a lower fertility index (67%). All other indices are 87 to 100%.

Conclusion. In this very limited reproduction test (one litter of one generation - parental rats fed only for 90 days preceding mating), 150 ppm DMCF is "no-effect."

Exhibit 4. Metabolism of oxamyl in tomato fruit. J. Harvey, Jr., Biochem, Dept., du Pont.

Oxamyl labelled with C^{14} was spotted on green tomato fruit on plants in a greenhouse. In ripe fruit at 14 days, oxamyl comprised 59% of radioactivity recovered; oxamyl oxime, 13%;

the glucose conjugate of oxamyl oxime (Metabolite A), 5%; DMCF, 4%; and polar metabolites, 19%. Results are said to resemble those previously obtained on treated apple or orange fruit.

Exhibit 5. Metabolism in the rat of N,N-dimethyl-1-cyanoformamide (DMCF), a metabolite of oxamyl. J. Harvey, Jr., Biochem. Dept., du Pont.

Method. A male Charles River CD rat, fed 450 ppm cold DMCF for one week, was given by stomach tube 1.1 mg (10.7 μCi) DMCF labelled in the cyano moiety with C^{14}. The rat was kept in a metabolism chamber for 72 hours; urine, feces, and expired air were collected. The rat was killed. Samples of freeze-dried liver, carcass, and hide, as well as samples of blood, urine, and feces, were processed to measure and characterize the C14 therein.

Results. There was a trace of radioactivity (0.3%) in expired air, identity of which (e.g., CO_2 or HCN ?) is not given. About 64% of radioactivity was in urine, and 5% was in feces. There was ca. 5% radioactivity in hide and carcass each; ca. 2% in G.I. tract, liver, and blood each; ca. 0.4% in kidneys and lungs each; less than 0.2% in heart, muscle, and spleen each; and less than 0.1% in testes, brain, and fat each. Total radioactivity recovered was 87%.

No free organosoluble compounds, including DMCF, were found in urine (< 0.5%). There were only small amounts of organosoluble (EtOAc-soluble) radioactive material in liver, carcass, hide, and blood - 2, 8, 0.4, and 2% respectively.

In urine, at least 22% of adminstered radioactivity was identified as conjugates of either the N,N-dimethyl or the N-methyl oxamic acid.

Another 27% of radioactivity in urine "behaved as carbon-14 incorporated into amino acids."

Results which are presumed to support this latter conclusion include:

1. Retention of part of a methanol extract of urine on a Dowex cation exchanger and subsequent partial elution with ammonium hydroxide;

2. Splitting into two fractions of that NH_4OH eluate (by high performance liquid chromatography on Aminex A-6 Ca++ resin), each of which gave one band on thin-layer chromatography on silica gel, which gave a purple color with ninhydrin reagent.

3. formation of two of the [illegible] spots (on thin-layer chromatography on cellulose of the methanol eluate of each band), each of which gave a color with ninhydrin.

The report (p.9) provides evidence that the oxamic acids were not tied up with the "amino acid" fraction. That is, a portion of the NH_4OH eluate (of the MeOH urine extract) when treated with $BCl_3/ClCH_2CH_2OH$ reagent, gave rise to no esters of either oxamic acid.

Rather, the (22%) oxamic acids found in urine occurred in that portion not retained on the column in step (1) above. They were identified as the 2-chlorethyl esters, formed by treating that portion with $BCl_3/ClCH_2CH_2OH$.

Tissue (liver, carcass, and hide) radioactive residues were extensively hydrolyzed and solubilized with Pronase® (a protein-[illegible] on the Dowex resin and then eluted by NH_4OH. Treatment of the ammonium hydroxide eluate to make trifluoroacetyl/o-butyl derivatives failed to identify any C^{14} containing amino acids supposedly there because of "low specific activity." Solubilized radioactivity which was not retained at all on the Dowex in step (1) was not retained on passage through an anion exchanger, either. It was concluded, therefore, that Pronase treatment of the rat tissues did not liberate any radioactive carboxylic acids such as the mono- or the dimethyl oxamic acid.

Blood fractions, solubilized with Pronase, chromatographed like amino acids, too. However, no radioactive ones could be identified due to low specific activity.

Comment. Neither for urine, nor for tissues or blood, did this study provide any positive proof of incorporation of radioactivity from [illegible]-labeled CMCF into amino acids contained therein. Evidence provided consists of chromatographic behavior, chiefly, and, therefore is not conclusive.

Exhibit 6. In vitro rat liver microsomal metabolism of oxamyl and selected metabolites of oxamyl. J. C-Y. Han and J. Harvey, Jr. Biochem. Dept., du Pont.

In vitro incubation of C^{14}- labelled oxamyl in presence of the 15,000 x g supernatant of a rat liver homogenate and co-factor (nicotinamide adenine dinucleotide phosphate; NADPH) for 2 hours at 37°C produced oxamyl oxime, DMCF, and N,N-dimethyloxamic acid as major metabolites. Minor metabolites (<5%) were the mono-methyl analogs of oxamyl and of oxamyl oxime. C^{14}-labelled DMCF, incubated similarly, gave only one metabolite, N,N-dimethyloxamic acid. C^{14}-labelled oxamyl oxime and the C^{14}-labelled monoglucose conjugate of oxamyl oxime (Metabolite A) treated similarly, each produced no (< 2%) metabolite.

Comment. Evidently, oxamyl is demethylated enzymatically, and either oxamyl or its monomethyl analog are converted enzymatically to DMCF or its monomethyl analog (and then to oxamic acids). In a second pathway of conversion, oxamyl is hydrolyzed to oxamyl oxime, evidently non-enzymatically; since a blank incubation mixture with oxamyl (containing no liver) produced as much oxamyl oxime as was produced in the presence of liver.

Exhibit 7. Eight-day dietary LC50-mallard ducks. Truslow Farms, Inc., 1/30/74.

"The acute LC_{50} of H-8541 'Vydate' is 5,025 ppm (confidence limits 1,460 to 17,295 ppm)."

Exhibit 8. Vydate fish toxicity evaluation, Mie Innerwater Fishery Exp. Station, ltr, to T. Asami from Y. Watanabe, du Pont Far East Inc., Japan, 12/9/75.

TLM values for carp are 29 ppm (active ingredient) on 1% granule product and 35 ppm (active ingredient) on technical material, each, for 48 hours of exposure. Hardly any survivors recovered physical strength; many died later. Therefore longer exposure would probably lower the TLM (and show greater acute toxicity).

Exhibit 9. Effect of pesticides on agriculture. E.L. Atkins et al., University of California at Riverside, Project No. 1499, 1974 annual report.

Oxamyl at 0.5 of 1 pound active/acre was, respectively, moderately and moderately-to-highly hazardous to bees.

Exhibit 10. "Vydate" - Alfalfa-Bee Study. Data from Dr. C. Johanson, Washington State University, undated.

Method. Alfalfa foliage was treated with oxamyl in cages and allowed to dry. Honeybees, alkali bees, and leafcutter bees were introduced, 3 hours later and 8 hours later. Bees foraged the area, 24 hours after time of release, and numbers of dead were counted.

Results. Mortality varied from 13 to 33% and from 3 to 12% among three kinds of bees which were introduced at 3 and 8 hours, respectively, to foliage pre-treated with 0.5 pound/acre of oxamyl (active ingredient). Mortality varied from 21 to 49% and from 11 to 19% among bees introduced at 3 and 8 hours, respectively, to foilage pre-treated with 1 pound/acre of oxamyl. Alkali bees appeared more susceptible than the other two strains of bees tested.

Exhibit 11. Bee research investigations, 1973. C. Johansen et al., Entomology Dept., Washington State University, Pullman, Washington.

Oxamyl a plied to a small area of alfalfa at 0.5 pound active/acre was moderate-to-low in hazard to three strains of bees; one pound/acre was high in hazard to two species, but not to the third.