UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

SUBJECT: Oxamyl, Petitioner's 6/8/76 reply to reject letter of 2/13/76 (Sanders-Mobley, du Pont) - TB evaluation of pertinent points, PP No. 6F1695.

DATE: NOV 18 1976

FROM: TB/RD

TO: Frank Sanders
Project Manager

PP No. 6F1695

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

Conclusions:

1) Deficiency 2 of the reject letter (memo title, above) is satisfied.
2) Deficiences 3 and 4 remain unsatisfied for lack of the required metagenicity and oncogenicity data.
3) Deficiencies 1 and 5 are not wholly satisfied. Petitioner is asked to answer the following:

a. What specific evidence is there for formation or lack of formation of conjugates of oxamyl or its metabolites in the liver or other body tissues of non-ruminant mammals? If they form, what is their chemical nature, and do they persist in body tissues?

b. Are plant polyglucose conjugates of oxamyl oximes cleaved and/or absorbed and metabolized by the non-ruminant animal (e.g., rat)? If so, to what extent?

c. In Exh. I, p. 14, 3rd para., this submission, some "tissues" are said to have contained no conjugated I, II, or DMCF. The tissues are not specified, and the "experimental" portion of Exh. I contains no mention of ethyl acetate extraction of any acid-hydrolyzed tissue. How does one explain this seeming discrepancy, and which of the "acid-hydrolyzed tissues" yielded "less than 3% radioactivity when extracted with ethyl acetate?"

Recommendation:

TB recommends that the requested tolerance (on potatoes at 0.1 ppm) for oxamyl not be established for reasons given above.

Introduction:

Items of the reject letter (memo title) pertinent to TB follow:
1) "....oxamyl metabolites formed in vivo by non-ruminants must be more completely characterized and the mode of binding determined if such exists. It must also be demonstrated whether the monoglucose or polyglucose conjugates of the oxime and demethylated oxime are absorbed and/or cleared from the G.I. tract when fed to non-ruminants...."

2) "F3b rat weanling histopathologic findings from the reproduction study already carried out is needed."

3) "A second lifetime feeding study, expecially examining for oncogenicity on significant components of oxamyl residues that would be found on the treated plants, is needed."

4) "Mutagenicity studies on significant components of oxamyl residues that would be found on treated plants are needed."

5) "More complete characterization of oxamyl metabolites formed in vivo by the non-ruminant mammal, including bound metabolites, and also, determination of the extent of oxalate formation-if any-is needed."

Petitioner's reply does not address the individual points of the reject letter. It does note, "The F3b rat weanling histopathologic findings from the reproduction study was submitted previously in the long-term rats and dogs feeding study (See Petition 3G1316, filed 9/22/72; H.L. 37-72, Project MR-1203, Part I, Feb. 2; see Summary, pages 7, 8, Tables XXII-XXIX)."

New Tox Data:

These comprise two rat studies using C14-labeled compounds, oxamyl and "Metabolite A" (methyl N',N'-dimethyl-N-[1-glucosyl)oxy]-1-thiooxamimidate) (Exhibits 1 and 3); a discussion of proof of absence of the oxamyl metabolite, DMCF, in the potato (Exhibit 4); and a study of metabolism of C^{14}-oxamyl in the lactating goat (Exhibit 5); and a study of potato metabolism of oxamyl, previously submitted and reviewed (Exhibit 2).) More details follow:

Exhibit 1. Metabolism of Oxamyl in the Rat. J. C-Y. Han, R.A. Jackson, and J. Harvey, Jr.

Procedure: Two adult male rats each received orally 1 mg oxamyl (methyl N',N'dimethyl-N-[(methylcarbamoyl)oxy]-1-thiooxamimidate), which contained C^{14} (the atom to which thio and imino groups are attached), after preconditioning with 50 or 150 ppm cold oxamyl in the diet. Each rat was placed in a metabolism cage, and expired air, urine, and feces were collected at intervals. Rats were killed at 72 hrs after dose.

Blood samples and tissues (brain, lungs, heart, liver, spleen, kidneys, testes, gastrointestinal (G.I.) tract, and portions of muscle and fat) were collected. Carcass minus hide was ground up. Fractions and samples collected were processed appropriately and radioactivity determined by liquid scintillation counting (lsc). Characterization of the radio-labelled compounds in various fractions was attempted.

Results: About 70% of radioactivity was excreted by each rat by 72 hrs, most of it in urine and none (< 0.3%) in respired air. About 18% was in hide, carcass, and G.I. tract. About 3% was in liver and blood. Remaining 1% accounted for was distributed evenly throughout other animal tissues.

On thin-layer chromatography (TLC) of fresh urine, no radioactivity migrated from the origin. Therefore, urine did not contain any of the following substances in free form: Oxamyl; its oxime (I); the N'-desmethyl oxime (II); N,N-dimethyl-1-cyanoformamide (DMCF); the S-oxide of oxamyl, the S-oxide of (I); the S,S-dioxide of oxamyl; or the S,S-dioxide of (I). In confirmation - each of these is soluble in ethyl acetate, but this solvent extracted less than 1% of radioactivity from either fresh urine or feces. Evidently, oxamyl is transformed and execreted in urine and feces only as very polar, water-soluble materials.

Gel permeation chromatography of urine showed the radioactive molecules to be larger than (I) and probably to be conjugates. Anion exchange chromatography showed them to be acidic in nature, suggesting glucuronides or sulfates, but treatment of urine with beta-glucuronidase-aryl sulfatase failed to liberate ethyl acetate-soluble radioactivity. However, acid (MeOH-HCl) did hydrolyze them, and TLC and GLC identified (I) and (II) as components of these conjugates. Cleavage by Lewis acid ($BCl_3/ClCH_2CH_2OH$) and TLC, corresponding identified N,N-dimethyloxamic acid (III) and N-methyloxamic acid (IV) as other components of the conjugates. About 75% of radioactivity of urine and feces was shown to be conjugates of these four oxamyl-related compounds.

Ethyl acetate extraction of blood, liver, skin, hair failed to remove radioactivity (< 1%); thus, neither oxamyl, (I), (II), nor DMCF were present in free form. Ethyl acetate extracted < 3% radioactivity from acid-hydrolyzed "tissues which contained....large amounts of radioactivity." Presumably, these compounds were not present in combined form, either, in the latter (unspecified) tissues. Note our question on this, under DISCUSSION.

About one-half of radioactivity originally present in rat skin/hair and rat blood was then shown to be incorporated into natural amino acids. [Samples were hydrolyzed (by refluxing with 6N HCl for 24 hrs), and amino acids were isolated (on an ion exchange column) and made into (N-butyl-trifluoroacety) derivatives, which were subjected to gas chromatography (GLC) (on a gas chromatograph equipped with a splitter, which sent part of the gas stream through for GLC analysis and retained

part for assay of radioactivity). This assay showed the radioactive substances to be amino acids, as did further GLC/mass-spectrum confirmation.]

Petitioner notes that, although remaining radioactivity in the tissues examined was not identified, "the fact that it (was) not found as known metabolites or oxamyl," together with the observed extensive labelling of normal amino acids, suggests that other natural products in tissues were also labelled with C^{14} from "complete breakdown" of the oxamyl molecule.

Exhibit 3. Metabolism of Metabolite A of Oxamyl in the Rat. J. Harvey, Jr.

Procedure: The preconditioning and dosage of the rat, confinement in metabolism cage, time of killing, and processing of tissues, fluids, and expired air was somewhat similar to that described under Exhibit 1, above.

Results: C^{14}-labelled "Metabolite A" (methyl N',N'-dimethyl-N-[1-glucosyl) oxy]-1-thiooxamididate), a major metabolite of oxamyl in potatoes, given orally to a rat, yielded 64% radioactivity in urine, 5% in feces, and none (0.1%) in expired air by 72 hrs. In urine, 30% or original dose was unchanged Metabolite A. In addition, there were conjugates of the oxamyl oxime (I), the N'-desmethyl oxime (II), N,N-dimethyloxamic acid (III), and N-methyloxamic acid (IV). No free DMCF, I, II, the S-oxide of I, the S,S-dioxide of I, nor any other organosoluble radioactive compounds were found in urine. About 4% of radioactivity or original dose occurred each in hide and carcass and 1.5% each in GI tract, liver, and blood. About 1% was distributed throughout the remaining animal tissues examined. Total recovery of radioactivity of original dose was 83%. Approximately one-half of radioactivity in liver, carcass, and blood was recovered in the amino acid fraction of enzyme (Pronase) hydrolyzed protein, and none was free I, II, or DMCF. Remainder of tissue radioactivity was not identified. Petitioner, however, suggests that tissue C^{14}-residue was incorporated into "many other natural products in tissues."

Exhibits 2, 4, and 5, of interest, chiefly, to CB, purport to show:

In potato, C^{14} residue consists chiefly of "non-toxic oxamino metabolites conjugated with glucose (....39%)," including Metabolite A, and as C^{14} reincorporated (via C^{14}-glucose) into starch (at least 35%) (Exhibit 2). No C^{14}-DMCF is found in potatoes, peanuts, or tobacco. (DMCF does occur in oranges and apples) (Exhibit 4).

Two lactating nanny goats, given C^{14}-oxamyl, excreted 60-70% radioactivity in urine and feces and only traces in expired air. No intact oxamyl or oximo compound was detected in milk. Radioactivity was found reincorporated into radioactive lactose (20%), casein (22%), and triglyceride fats (7%), and, presumably, other milk proteins (6%). Two unidentified components of milk (21% and 7% of total C^{14}) did not appear to have an oxamyl moiety. Blood and tissues had about on-half of radioactivity present in

protein, probably as amino acids - but not positively identified as such, and none as oxamyl or oximino compounds. Petitioner says that C^{14} enters the metabolic pool and forms many natural components (Exhibit 5).

Old Tox Data:

In the previously submitted rat reproduction study, histopathologic findings on F3b weanlings (which we had asked Petitioner for) are negative. Two male and two female pups from each of five litters of the control and top-dose (150-ppm) groups were evaluated.

Discussion:

Deficiency 2 of (7/13/76) reject letter, this PP, is now satisfied, but deficiences 3 and 4 remain unsatisfied.

Petitioner's rat metabolite studies on oxamyl (Exh. 1) and Metabolite A (Exh. 3) give some, but not wholly satisfying, information on Deficiencies 1 and 5. We elaborate on this below:

Exhibit 1, this PP, now shows that most oxamyl is fairly rapidly excreted in urine and feces of the rat as polar, water-soluble conjugates of the oxamyl oxime (I), its desmethyl oxime (II), and the two oxamic acids (III and IV). What these oxamyl metabolites are conjugated with is unknown. It is suggested that the conjugates are acidic, but they were not cleaved by glucuronidase or aryl sulfatase.

It is noteworthy that neither the sulfone nor the sulfoxide of oxamyl or its oxime was excreted in urine. In contrast, the rat metabolizes aldicarb [2-methyl-2(methylthio)propionaldehyde-O-(methylcarbamoyl)oxime], a pesticide structurally related to oxamyl, to both the sulfoxide and the sulfone, which are potent anticholinesterase metabolites, and these latter are excreted in urine (Dorough, 1970).

In tissues examined (skin, hair, liver, blood), 72 hrs after dose, no free oxamyl or free oximes (I or II) or free DMCF occurred.

In some "tissues" no conjugated I, II, or DMCF are said to have occurred, either (Exh. 1, p. 14, 3rd para.). The tissues are not specified, and the "experimental" portion of Exh. 1 contains no mention of ethyl acetate extraction of any acid-hydrolyzed tissue. Petitioner is asked to explain this seeming discrepancy and, also, to specify which one of the "acid-hydrolyzed tissues" in Exh. 1 yielded "less than 3% radioactivity when extracted with ethyl acetate" (Exh. I, this PP, p. 14, para. 3).

In skin, hair, and blood, about one-half of radioactivity was identified as amino acids; like those which occur naturally in protein. Thus part of oxamyl is degraded and transformed into naturally-occurring substances.

Liver, however, was not further examined. We presume from results of in vitro tests made previously (cf. our memo of 1/6/76, this PP) that oxamyl was metabolized in liver to I, II, III, and IV. These could then have been conjugated in liver and either stored and later excreted or excreted directly in urine and feces. However, no direct proof of either possibility is provided.

We would like to have had liver further examined for any conjugates of oxamyl metabolites which may have formed there, but this was not done. Nor were the execreted oxamyl-derived conjugates identified; although they appear not to have been glucuronides or sulfates - such other as carbamate pesticides often form (Dorough, 1970). Exhibit I, therefore, is judged deficient in showing where - in rat metabolism - oxamyl metabolites conjugate and with what. And a discrepaney in Exhibit I - as to what, if any, acid-hydrolyzed tissues were checked for presence of oxamyl metabolites (by ethyl acetate extraction) - needs to be explained (cf. above).

Metabolite A, an oxamyl oxime glycoside formed in potatoes, contains one glucose residue/molecule; although most such glycosides contain poly-glucose residues. Potatoes, treated with oxamyl according to label directions, could have as little as 0.03 ppm free oxamyl plus free oximes along with ca. 0.5 ppm of these glycosides, i.e., more than 90% as glycosides (CB memo of 1/30/76, this PP, and personal communication of E. L. Gunderson, CB, to M. Quaife, 11/2/76). Treated peanuts, tobacco, apples, and oranges also have sizable proportions of oxime glycosides (CB memo of 1/30/76, PP No. 6F1696). The proposed enforcement method determines only the free oxime plus free oxamyl. The metabolic disposition of the glycosides and toxicity to humans, if any, is of concern.
Exhibit 3 now shows that one-third of Metabolite A was excreted by the rat unchanged.

Exhibit 3 shows that the remainder of Metabolite A was metabolized. Part was degraded to desmethyl oxime and/or hydrolyzed to dimethyl and/or monomethyl oxamic acid and excreted in urine or feces as conjugates. Part, e.g., in liver, was incorporated into a radioactive hydrolyzed protein fraction, presumed - but not proven - consist of radioactive amino acids. The remaining one-half of liver radioactivity was not characterized; although free oxamyl, I, II, and DMCF were excluded as present. In summary, that part of the monoglucose conjugate of oxamyl oxime (Metabolite A) which was absorbed by the rat was metabolized like the free oxamyl oximes; so it should have the same degree (or lack) of toxicity as they have.

Petitioner provides no information as to whether the polyglucose conjugates of oxamyl oximes are absorbed and/or metabolized. Nor does Exh. 3 show whether any bound metabolites were formed from Metabolite A in liver or other tissues, and, if so, their chemical nature or whether they persist in tissues.

These oximes and their glycosides could be relatively innocuous. [For example, the beta-glycoside of 4-hydroxy-1-naphthyl N-methylcarbamate is acutely only about one-thirtieth as toxic as its aglycone (given to mice, i.p.) (Cardona and Dorough, 1973).] The free oxamyl oxime killed no rats when given p.o., daily for ten days, at 1g/kg BW (cf. our memo of 10/20/75, pp No. 1650). On the other hand, the oxamyl oximes, conceivably, form conjugates in animals (as they do in plants) which might be biologically active. (For example, the oxime, 2-pyridine aldoxime methiodide (2-PAM), counteracts organophosphate pesticide poisoning by hastening dephosphorylation of acetylcholinesterase (Casarett and Doull, 1975).] TB reiterates need for further information from Petitioner on points mentioned in "Discussion," above.

Possible oxalate formation from oxamyl and its free or conjugated metabolites is now judged not of toxicologic concern. Human consumption of oxamyl and free or conjugated oximes on r.a.c.'s at a rate compatible with the tentative ADI for oxamyl (1.5 mg/day fro 60-kg adult) should not provide harmful intake of oxalate; since the metabolic fate of at least three-fourths of oxamyl free and bound plant metabolites is shown to be otherwise - in mammalian metabolism.

References:

1) Dorough, H. W., 1970. "Symposium on Metabolism. Metabolism of Insecticidal Methylcarbamates in Animals," J. Agr. Food Chem 18, 1015-22.

2) Car ona, R. A., and H. W. Dorough, 1973. "Syntheses of the Beta-D-Glucosides of 4- and 5-Hydroxy-1-naphthyl N-Methylcarbamate," J. Agr. Food Chem. 21, 1065-71.

3) Casarett, L. J., and J. Doull, eds., 1975. "Toxicology, the Basic Science of Poisons," MacMillan Publishing Co., Inc., N.Y., p. 424.

MLQ. 11/17/76

Mary L. Quaife, Ph.D., TB/RD
November 3, 1976

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