PmSD/ISB

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

FEB 13 1990

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: A/S Cheminova: Response to the Ethyl Parathion Reregistration Standard: Residue Chemistry Data (MRID # 41228601, DEB # 6010.)

FROM: R. B. Perfetti, Ph.D., Chemist
Reregistration Section
Dietary Exposure Branch
Health Effects Division (H7509C)

R B Perfetti

THRU: W. J. Boodee, Section Head
Reregistration Section
Dietary Exposure Branch
Health Effects Division (H7509C)

TO: Reto Engler, Ph.D., Chief
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

and

J. Talarico, RM-74
Reregistration Branch
Special Review and Reregistration Division (H7508C)

Attached is the review of residue chemistry data submitted by A/S Cheminova in response to the ethyl parathion reregistration standard. This information was reviewed by Dynamac Corporation under supervision of Dietary Exposure Branch, HED.

These studies have undergone secondary review in Dietary Exposure Branch and have been revised to reflect the Branch policies.

If you need additional input please advise.

Attachment 1: Review of Ethyl Parathion Residue Chemistry Data

cc: With Attachment 1: R.B. Perfetti, (TOX), J. Burrell (FOD), Ethyl Parathion Reregistration Standard File, Ethyl Parathion Subject File, J. Talarico (RB, SR&RD)

cc: Without Attachments: P. Fenner-Crisp (HED), M. Hawkins (HED), Circulation (7), RF, R. Engler (SACB)

H7509C:DEB:X77484:CM#2:RM810:R.B.Perfetti:2/13/90

DYNAMAC
CORPORATION

Final Report

ETHYL PARATHION
Task 4. DEB No. 6010. Registrant's Response to Residue Chemistry Data Requirements

FEBRUARY 9, 1990

Contract No. 68-D8-0080

Submitted to:
Environmental Protection Agency
Arlington, VA 22202

Submitted by:
Dynamac Corporation
The Dynamac Building
11140 Rockville Pike
Rockville, MD 20852

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ETHYL PARATHION

Registrant's Response to Residue Chemistry Data Requirements

Task - 4

BACKGROUND

The Ethyl Parathion Guidance Document dated 12/15/86 identifies numerous residue chemistry data requirements, including data on the qualitative nature of the residue in or on wheat grain and forage resulting from foliar applications of ethyl parathion. A/S Cheminova responded to this data requirement by submitting one volume of data (MRID 40751601) pertaining to the metabolism of [^{14}C]ethyl parathion in wheat. These data were reviewed by the Agency (DEB No. 4224, 11/29/88) and it was concluded that additional data are required depicting the identification of residues and a materials balance of [^{14}C]-residues for each commodity. In addition, it was concluded that the registrant must provide an adequate explanation for the observed radioactive residues in plants harvested 1 day prior to application of the test material. The Registrant has submitted one additional volume (DEB No. 6010, MRID 41228601) containing additional metabolism data on wheat, which are discussed in this review.

DEFICIENCIES REMAINING TO BE RESOLVED

Data have been submitted in response to the requirement for metabolism data on ruminants and poultry (MRIDs 40388901, 40388902, 40623801, and 40784401), and have been found insufficient by the Agency (DEB Nos. 2644, 10/29/87; 3935, 9/29/88; and 4302, 1/30/89). The requirement for tobacco pyrolysis data is currently reserved pending the outcome of tobacco residue studies (DEB No. 6032, 11/29/89). The remaining residue chemistry data gaps on plant metabolism (cotton and potatoes); storage stability; residue data on beets, carrots, potatoes, radishes, rutabagas, sugar beets, sweet potatoes, turnips, beet tops, sugar beet tops, turnip tops, garlic, onions, celery, lettuce, spinach, broccoli, cabbage, kale, kohlrabi, beans (dry and snap), peas, bean vines and hay, pea vines, soybean hay, peppers, tomatoes, cucumbers, melons, summer squash, winter squash, grapefruit, lemons, oranges, apples, pears, apricots, plums, cherries, blackberries, cranberries, grapes, strawberries, almonds, pecans, walnuts, field corn, sweet corn, oats, rice, sorghum, corn forage and fodder, oat forage, hay and straw, rice forage and straw, sorghum forage and fodder, grass forage, clover, alfalfa, artichokes, avocados, cottonseed, dates, figs, hops, mangoes, okra, olives, peanuts, pineapples, rapeseed, sunflowers, and tobacco; and processing data on potatoes, sugar beets, citrus, tomatoes, apples, prunes, grapes, corn, oats, rice, sorghum, cottonseed figs, hops, peanuts, pineapples,

rapeseed, and sunflower seed that were identified in the Guidance Document have not been addressed and are still unresolved.

The Conclusions and Recommendations stated below apply only to the wheat metabolism data contained in this submission. The other data requirements stated above remain outstanding.

CONCLUSIONS

1. The Registrant has not provided an explanation for the observed radioactive residues in plants harvested 1 day prior to application of the test material (MRID 40751601); this issue remains unresolved.

2. The nature of the residue in on or wheat grain and straw is inadequately defined because only 65% of the total radioactive residue (TRR) in grain and 54% of the TRR in straw, consisting of unchanged ethyl parathion and S-phenyl parathion, were adequately characterized and quantified. One additional metabolite, p-nitrophenol, was identified but not adequately quantified; this compound and seven other inconclusively identified compounds together constituted 19 and 16% of the TRR in grain and straw, respectively. Bound residues not released by mild acid hydrolysis accounted for 17% of the TRR in straw. Additional attempts should be made to identify and quantify the tentatively characterized compounds in grain and straw and to release and identify the remaining bound residues in straw.

RECOMMENDATIONS

The registrant should be informed that additional attempts should be made to conclusively characterize and quantify those compounds isolated from grain and straw that were only tentatively identified and/or inadequately quantified. We recommend that additional solvents be used in analysis of ^{14}C-residues by thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC). In addition, the registrant should attempt to release and characterize additional bound residues in wheat straw using enzyme digestion or more rigorous chemical hydrolysis procedures. Finally, the registrant should be instructed to explain the presence of radioactive residues in samples collected prior to treatment (MRID 40751601).

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DETAILED CONSIDERATIONS

Total Radioactive Residues (TRR)

Wheat plants were given two foliar applications (7 days apart) of uniformly ring-labeled [^{14}C]ethyl parathion (specific activity at application: 2.1 mCi/mmole; radiochemical purity: >95%) at a rate of 1.2 lb ai/acre at each application (ca 1.5x the maximum registered rate). Straw, chaff, and grain were sampled 1, 3, and 7 days following each application. (Refer to the Agency review of MRID 40751601, DEB No. 4224, 1/29/88, for details of the application and sampling protocol).

The total radioactive residues (TRR) in or on wheat commodities at each sampling interval were determined by combustion/liquid scintillation spectrometry (LSS); the results are summarized in Table 1.

Table 1. Total radioactive residues (ppm ethyl parathion equivalents) in or on wheat commodities harvested following two foliar application using [^{14}C]ethyl parathion (data from MRID 40751601).

Interval (days)	Commodity		
	Chaff	Grain	Straw
1	65.9-169	1.36-5.15	20.2-46.5
3	85.8-107	1.61-3.66	24.4-35.7
7	61.7-74.6	3.43-5.08	21.2-21.8
8	164-178	3.31-6.03	69.9-79.4
10	111-188	2.95-9.77	46.6-78.2
14	234-318	6.70-10.2	85.6-111

The TRR in samples of wheat straw, chaff and grain that were used for metabolite identification were reported in the current submission to be 129 ppm in straw, 314 ppm in chaff, and 10.4 ppm in grain. The discrepancies between the maximum TRR reported for grain and straw in MRID 40751601 and the TRRs of the samples used in this study were not explained.

Extraction and Hydrolysis

Samples collected on the last sampling date and stored for an unspecified period of time were used for further analysis. Subsamples of wheat straw, chaff, and grain were extracted using methanol:water (80:20 v/v), filtered, and partitioned with chloroform and additional water to yield aqueous and organosoluble phases plus an unextracted residue. The unextracted residue was subjected to mild acid hydrolysis by refluxing in 1% hydrochloric acid in 95% methanol for 30-40 minutes. After refluxing, the hydrolysate was partitioned with

chloroform to yield aqueous and organosoluble phases plus an unextracted residue. Radioactivity in the extracts was determined by liquid scintillation spectrometry (LSS) and in insoluble residues by combustion/LSS.

Distribution of radioactivity in residue fractions before and after hydrolysis is presented in Table 2. At least 57.1% of the TRR in straw, 72.7% in chaff, and 76.6% in grain was extractable initially. Non-extracted residues accounted for 32.7% of the TRR in straw, 25.0% in chaff, and 17% in grain. Up to 10.2% of the TRR in straw was unaccounted for.

Table 2. Percent distribution of radioactivity in fractions from wheat commodities harvested following two foliar applications of [^{14}C]ethyl parathion (data from MRID 41228601).

	% of TRR		
Fraction	Straw	Chaff	Grain
Extracted			
Organosoluble	52.0	62.5	64.8
Aqueous	5.1	10.2	11.8
Total extracted	57.1	72.7	76.6
Non-extracted	(32.7)	(25.0)	(17.0)
Hydrolyzed[a]:			
Organosoluble	16.7	12.4	11.2
Aqueous	1.0	1.9	0.7
Unextracted	15.0	10.7	5.1
Total recovered	89.8	97.7	93.6
Unaccounted	10.2	2.3	6.4

[a] The data reported in the submission were from radioassays of the individual fractions after hydrolysis; for this review, these values have been normalized to reflect percentages of the original non-extracted fraction; the percent of the TRR that was initially unextractable appears in parentheses.

Characterization of Residues

Aqueous and organosoluble residues in extracts obtained before and after hydrolysis were analyzed using thin-layer chromatography (TLC) and/or high performance liquid chromatography (HPLC). The solvent systems used for TLC were petroleum ether:diethyl ether:acetic acid (50:45:5, v/v) and water:acetonitrile:methanol (50:25:25, v/v). Residues were eluted from HPLC using a stepwise gradient of 100% 0.02 M ammonium acetate to 100% acetonitrile. Compounds were identified by co-chromatography with standards (TLC) or by comparison with

elution times of standards (HPLC). Radioactivity on TLC was quantified by scanning; fractions collected from HPLC were radioassayed using LSS.

Ethyl parathion and nine of its metabolites were conclusively or tentatively identified by TLC and/or HPLC. However, only ethyl parathion, p-nitrophenol and S-phenyl parathion, were identified by both TLC and HPLC, and the parent compound and S-phenyl parathion were quantified unambiguously by both methods. The remaining seven putative metabolites [ethyl paraoxon, O-ethyl-p-nitrophenyl phosphorothioate (ENPP), p-nitrophenyl-β-D-glucopyranoside (NPGP), S-ethyl parathion, p-aminoparathion, p-aminophenol, and p-nitrophenyl phosphate (NPP)] could be identified by one of the two methods but not by both. HPLC resolved ethyl paraoxon and NPGP, but not S-ethyl parathion from p-aminoparathion; p-nitrophenol could be resolved from organic extracts, but co-eluted with ENPP in aqueous extracts, and, therefore could not be accurately quantified. NPP eluted in the void volume from HPLC and p-aminophenol was unstable under HPLC conditions. TLC, on the other hand, resolved S-ethyl parathion, p-aminoparathion, and p-nitrophenol, but NPP in some extracts migrated with the solvent front, and the remaining five compounds were either poorly resolved or not at all.

Table 3 presents the relative amounts of ethyl parathion and S-phenyl parathion and the tentatively identified metabolites, and in addition, the amounts of uncharacterized radioactive residues in wheat straw, chaff, and grain. Unchanged ethyl parathion and S-phenyl parathion were extracted from all wheat matrices, and accounted for 53.8% of TRR in straw, 65.0% in chaff and 65.3% in grain. The tentatively identified and/or quantified metabolites (i.e. the seven putative metabolites and p-nitrophenol) amounted to 14.6% of the TRR in straw, 18.1% in chaff, and 21.6% in grain. The three unknowns accounted for 3.3%, 1.1%, and 0.4% of the TRR in straw, chaff, and grain, respectively.

Table 3. Radioactive residues in wheat straw, chaff, and grain (data are from HPLC analysis, unless otherwise indicated).

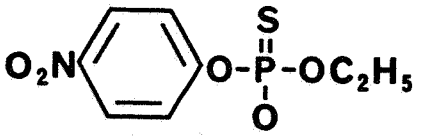
	Straw		Chaff		Grain	
Metabolite	%TRR	ppm[a]	%TRR	ppm	%TRR	ppm
Identified						
Ethyl parathion	51.3	66.2	62.4	195.9	64.6	6.72
S-Phenyl parathion	2.5	3.2	2.6	8.2	0.7	0.07
Total	53.8	69.4	65.0	204.1	65.3	6.79
Tentatively identified						
p-nitrophenol	5.4	7.0	3.9	12.2	6.1	0.63
ethyl paraoxon	3.3	4.3	3.7	11.6	1.2	0.12
ENPP	1.1	1.4	4.0	12.6	4.1	0.43
NPGP	1.1	1.4	3.2	10.0	4.6	0.48
p-aminophenol	2.3	3.0	1.3	4.0	1.0	0.10
s-ethyl parathion[b]	0.7	0.9	1.5	4.7	0.7	0.07
p-aminoparathion[b]	1.0		1.1	3.5	1.0	0.10
p-nitrophenyl phosphate[b]	0.7	0.9	1.7	5.3	0.3	0.03
Total	15.6	20.1	20.4	59.9	19.1	1.99
Uncharacterized						
Unknown (three HPLC peaks)	3.3	4.3	1.1	3.5	0.4	0.04
Not analyzed	1.1	1.4	2.0	6.3	0.8	0.08
Unextracted	17.0	21.9	11.5	36.1	5.5	0.57
Unexplained loss	10.2	13.2	2.3	7.2	6.4	0.67
Total	31.6	40.8	16.9	53.1	13.1	1.36

[a] Calculated using TRR values of 129 ppm for straw, 314 ppm for chaff, and 10.4 ppm for grain.

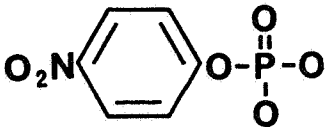
[b] Data are from TLC analyses.

The molecular structures of ethyl parathion and several metabolites are depicted in the Ethyl Parathion Residue Chemistry Chapter dated 4/8/85. Additional metabolites conclusively or tentatively identified in the current submission are illustrated below:

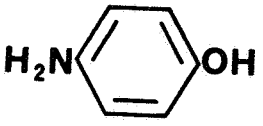
O-ethyl-p-nitrophenyl phosphorothioate



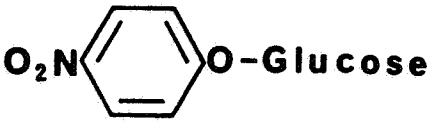
p-nitrophenyl phosphate



p-aminophenol



p-nitrophenyl-β-D-glucopyranoside



In summary, these data are insufficient to fulfill the requirement for metabolism data on wheat because only 65% of the total radioactive residue (TRR) in grain and 54% of the TRR in straw, consisting of unchanged ethyl parathion and S-phenyl parathion, were adequately characterized and quantified. One additional metabolite, p-nitrophenol, was identified but not adequately quantified; this compound and seven others inconclusively identified together constituted 19 and 16% of the TRR in grain and straw, respectively. Bound residues not released by mild acid hydrolysis accounted for 17% of the TRR in straw. Additional attempts should be made to identify and quantify the tentatively characterized compounds in grain and straw and to release and identify the remaining bound residues in straw. We note also that the registrant has not provided an explanation (requested in DEB No. 4224, 11/29/88) regarding the presence of radioactivity in plant samples taken prior to treatment reported in MRID 40751601.