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

FEB 8 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Diazinon - Ciba-Geigy's Response to the Data Call-In Notice (MRID Nos. 408798-01, -02, -03, -04, and -05 - DEB Nos. 4773, 4774, 4778, 4779, and 4780)

FROM: R.B. Perfetti, Ph.D., Chemist
Dietary Exposure Branch
Health Effects Division (TS-769C)

TO: George LaRocca, PM 15
Insecticide-Rodenticide Branch
Registration Division (TS-767C)

and

Reto Engler, Ph.D., Chief
Science Analysis and Coordination Branch
Health Effects Division (TS-769C)

THRU: W.J. Boodee, Section Head
Reregistration Section
Dietary Exposure Branch
Health Effects Division (TS-769C)

In response to a Data Call-In Notice for diazinon dated May 8, 1987, Ciba-Geigy Corporation has submitted metabolism studies on goats and laying hens dated November 4, 1988 (cover letter).

These studies and our conclusions regarding them are discussed below.

Conclusions

1. The metabolism of diazinon in goats involves dephosphorylation or desulfurylization with subsequent oxidation of alkyl side chains and conjugation with glucuronide species. The metabolism of diazinon in goats is adequately understood. The major portion of the terminal residue in milk and tissues will consist of diazinon, GS-31144, G-27550, G-24576, and CGA-14128.

2. The metabolism of diazinon in laying hens is not adequately delineated due to incomplete characterization of the terminal residue in tissues and eggs (< 30 and < 40 percent, respectively). An additional study in hens is needed. The study should provide a more complete characterization of radioactive residues in eggs and tissues.

   The registrant is encouraged to discuss the protocol for this additional study with the Dietary Exposure Branch before initiating any experiments.

The data requirement regarding the need for a ruminant metabolism study for diazinon is fulfilled. The requirement for a poultry metabolism study remains outstanding.

Discussion of the Data

Ciba-Geigy submitted a metabolism study (MRID Nos. 408798-04, 408798-05, and 408798-01) on lactating goats. The experiment involved oral dosing of two goats for four consecutive days with capsules of ringlabeled ^{14}C-diazinon at a level of ca. 100 ppm in the diet. Urine, feces, and milk were collected daily. Blood was sampled on days 2 and 4 of medication. The animals were sacrificed 24 hours after the last dose and samples of liver, kidney, tenderloin, leg muscle, omental fat, and perirenal fat were taken.

Feces and tissue samples were homogenized and these, along with blood samples, were combusted to determine radioactivity levels. Radioactivity in urine and milk samples was determined via liquid scintillation. Urine (approximately 64%) and feces (approximately 10%) contained the major portion of total radioactivity (72 to 76%). Radioactive residues in blood (0.5% of total dose) and milk (< 0.5% of total dose) ranged from 0.139 to 0.638 ppm and 0.2 to 0.687 ppm. Tissues contained 0.112 (perirenal fat) to 3.019 (kidney) ppm of radioactivity with essentially no difference in levels in muscle vs. fat. Highest levels of radioactive residues were observed in liver and kidney. Total radioactivity in all tissues ranged from 0.44 to 1.35 percent of total dose. Radioactivity plateaued in milk after ca. 3 days of dosing.

Goat urine was partitioned three times with 1:1 butanol:water. The aqueous fraction was then partitioned three times with ethyl acetate. The water fraction was concentrated and the butanol and ethyl acetate organic fractions were dried, concentrated, and combined. The organic and aqueous fractions were then dissolved in water, cleaned-up on a DEAE Sephadex column and analyzed via TLC.

Homogenized feces samples were extracted with 9:1 methanol:water, concentrated, then partitioned with butanol:water (1:1) and then ethyl acetate. The organic phases were then dried and combined, concentrated, cleaned-up and analyzed as discussed above for the organic extract of urine.

Milk samples were blended with acetonitrile, the solvent was stripped off and the sample was partitioned with hexane:water (1:1). The hexane phase was dried. The aqueous phase was partitioned with butanol followed by ethyl acetate. The samples were then handled as above.

Tissues were homogenized, extracted with methanol:water (9:1), and methanol was evaporated from the resulting extractable fraction. The remaining aqueous solution was partitioned with hexane and the organic fraction was dried and analyzed via TLC. The aqueous phase was sequentially partitioned with butanol:water (1:1), ethyl acetate:water (1:1), and the organic phases were combined, dried, cleaned-up, and analyzed via TLC. The aqueous fractions were combined and "cleaned up" on a C-18 Bond Elut column, concentrated and also analyzed via TLC.

Whole extracts from certain tissues were hydrolyzed with 6 N HCl overnight, neutralized, "cleaned up" on a Sephadex column, and analyzed via TLC.

Selected urine, kidney, and liver fractions were treated with bovine liver beta-glucuronidase worked-up as above and analyzed via TLC.

The following compounds were identified in goat urine: diazinon, 2-(alpha-hydroxyisopropyl)-6-methyl-4(1H)-pyrimidone (GS-31144), 2-isopropyl-6-methyl-4(1H)pyrimidone (G-27750), O,O-diethyl O-[2-alpha-hydroxyisopropyl)-4-methyl)-6-pyrimidinyl]phosphorothioate (CGA-14128) and O,O,-Diethyl O-[6-methyl-2-(1-methylethyl-4-pyrimidinyl] phosphate (G-24576).

In all, < 30 percent of the radioactivity in urine was identified with the major metabolites being GS-31144 and G-27550 either free or conjugated. The same compounds observed in urine were found in feces. Approximately 50 percent of the radioactivity in feces was identified with the major components again being GS-31144 and G-27550.

Radioactivity identified in milk consisted of 32.3 percent GS-31144, 39.3 percent G-27550 and 0.2, 0.1, and 0.2 percent G-24576, CGA-14128, and diazinon, respectively. In all, ca. 77 percent of the radioactivity in milk was characterized.

Radioactive residues in liver were identified as GS-31144 (19%), G-27550 (19.2%), G-24576 (0.3%), CGA-14128 (6.2%), and diazinon (0.2%). A total of ca. 30 percent of the unconjugated radioactivity in liver was characterized.

Radioactive components identified in kidney and various muscle tissues totaled 50 to 78 percent with essentially the same profile of amounts of each metabolite. Fat samples contained mainly diazinon (64 to 68%) with large percentages (12 to 13%) of CGA-14128. Metabolites observed in other tissues were also seen in fat but at much lower levels. A total of ca. 86 to 100 percent of the radioactivity in fat was characterized.

Structural assignments for metabolites in urine, feces, milk, and tissues were further confirmed utilizing TLC, GC, HPLC, GC/MS, and LC/MS chromatographic methods. Radioactivity profiles of various sample types indicated that metabolite identities and ratios were similar in all components.

The metabolism of diazinon in goats involves dephosphorylation or desulfurylization with subsequent oxidation of alkyl side chains and conjugation with glucuronide species. The metabolism of diazinon in goats is adequately understood. The major portion of the terminal residue in milk and tissues will consist of diazinon, GS-31144, G-27550, G-24576, and CGA-14128.

A metabolism study on laying hens (MRID Nos. 408798-01, -02, and -03) was also submitted. The experiment involved oral dosing of four laying hens for 7 consecutive days with capsules of ring-^{14}C-labeled diazinon at a level of ca. 20 ppm in the diet. Excreta and eggs were sampled daily. The animals were sacrificed approximately 24 hours after the last dose and samples of various tissues and blood were taken. Eggs were divided into whites and yolks and the shells were discarded.

Samples of excreta, tissue, and blood were homogenized and combusted to determine radioactivity levels. Radioactivity levels in egg whites and yolks were determined using liquid scintillation counting. Excreta contained the major portions (approximately 79%) of the total dose. Radioactive residues in blood (up to 0.03% of total dose) and whole eggs (0.07% of total dose) ranged from 0.084 to 0.257 ppm and 0.03 to 0.066 ppm, respectively. Residues in yolks and whites ranged from 0.006 to 0.065 ppm and from 0.038 to 0.066 ppm, respectively. Tissues contained 0.01 ppm (fat) to 0.148 ppm (kidney) of radioactive residues. Essentially no difference in residue levels was observed in muscle vs. fat. With the exception of the level of radioactivity observed in

eggs on day 7, a plateau was reached in whole eggs after 3 to 4 days as was found in milk. We find the somewhat higher radioactive residue observed in whole eggs on day 7 to be anomalous probably due to the low level of radioactivity involved in the eggs.

Excreta was homogenized, extracted with methanol:water (9:1). The methanol was evaporated and the sample was partitioned with butanol followed by ethyl acetate. The organic fractions were dried and combined, concentrated, cleaned-up on a DEAE Sephadex column, reconcentrated, and analyzed via TLC. The aqueous phases were concentrated and cleaned-up on a C-18 Bond Elut column, reconcentrated and also analyzed via TLC. An aliquot of the aqueous phase was also incubated with bovine liver beta-glucuronidase. After centrifugation, the resulting solution was cleaned up on a DEAE column as above and analyzed via TLC.

Homogenized samples of selected tissues, egg yolks, and egg whites were initially extracted with hexane:water (1:1) followed by further work-up and analysis as for excreta above. Tissue samples (after the hexane extraction) were hydrolyzed with 6 N HCl overnight, neutralized, cleaned-up on a DEAE Sephadex column, and analyzed via TLC.

The following compounds were identified in hen excreta: diazinon, 2-(alpha-hydroxyisopropyl)-6-methyl-4 (1H)-pyrimidone (GS-31144), 2-isopropyl-6-methyl-4(1H) pyrimidone (G-27550), O,O-diethyl O-[2-alpha-hydroxyiso-propyl)-4-methyl)-6-pyrimidinyl] phosphorothioate (CGA-14128) and O,O-Diethyl O-[6-methyl-2-(1-methylethyl-4-pyrimidinyl] phosphate (G-24576).

A total of ca. 37 percent of the total radioactivity in hen excreta was identified as GS-31144 (11.4%), G-27550 (6.3%), G-24576 (1.3%), CGA-14128 (1.7%) and diazinon (approximately 15%).

Radioactivity identified in egg whites consisted of 33.3 percent of GS-31144, 9.4 percent of G-27550, 1.3 percent of G-24576, 0.05 percent of CGA-14128, and 0.03 percent of diazinon. In all, ca. 45 percent of the total radioactivity in whites was characterized. Egg yolks contained GS-31144 (18.6%), G-27556 (11.1%), G-24576 (0.4%), CGA-14128 (0.06%), and diazinon (0.02%). A total of ca. 32 percent of the total radioactivity in yolks was identified.

Radioactive compounds identified in various tissues and fat given in decreasing order of abundance were GS-31144, G-27550, G-24576, CGA-14128, and parent. Percentages of total radioactivity characterized in the various tissues ranged from approximately 15 to 25 percent.

Structural assignments for metabolites in excreta, eggs, and tissues were further confirmed utilizing TLC, GC, HPLC, GC/MS, and LC/MS chromatographic methods.

The metabolism of diazinon in laying hens is not adequately delineated due to incomplete characterization of the terminal residue on tissues and eggs (< 30 and < 40 percent, respectively). An additional study in hens is needed. The study should provide a more complete characterization of radioactive residues in eggs and tissues. (Note: A major portion of the radioactive residue was observed in fraction S-12.)

The registrant is encouraged to discuss the protocol for this additional study with Dietary Exposure Branch before initiating any experiments.

TS-769C:DEB:RBP:2/89:CM2:RM812:x4381
cc:RF,Circ.,Perfetti,Reg.Std.Files,PMSD/ISB,A.Rispin(SIPS),
A.Kocialski(SACB)
RDI:W.Boodee 2/89;E.Zager,2/89