PMSD/ISB

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

FEB 14 1990

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Diazinon - Ciba-Geigy's Response to the Data Call-In Notice (MRID 412259-01. DEB No. 6034)

FROM: William O. Smith, Ph.D., Chemist
Reregistration Section
Dietary Exposure Branch (DEB)
Health Effects Division (H7509C)

W. O. Smith

TO: George LaRocca, PM 15
Insecticide-Rodenticide Branch
Registration Division (H7505C)

and

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

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

WJB

In response to a Dietary Exposure Branch review of a poultry metabolism study submitted in response to the Diazinon Data-Call-In notice, Ciba-Geigy has provided additional information on the nature of the radioactivity in poultry tissue and eggs. These data and our conclusions are discussed below.

Conclusions

The nature of the residue in poultry tissue and eggs is adequately understood. The pathway for catabolism of diazinon in poultry is similar to that in goats. The major portion of the terminal residue will consist of parent, GS-31144, G-27550, G-24576, CGA-14128, 2-(beta-hydroxy-isopropyl)-6-methyl-4(1H)-pyrimidinone, its glucuronide conjugate, and glucuronide conjugates of G-27550 and GS-31144 (Note: See our original review of 2/8/89 by R.Perfetti for the chemical names of compounds).

Discussion of the Data

Ciba Geigy, Corp. has submitted a poultry metabolism study (MRID 412259-01) entitled "Supplement to metabolite identification in hens and goats treated with ^{14}C-Diazinon". This study is an extension of a previously submitted metabolism study on laying hens (MRIDs 408798-01, 02, and -03), which was the subject of a review by R. Perfetti dated 2/8/89.

The study involved oral dosing of four laying hens for seven 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.

Extraction and characterization of soluble residues: Excreta were homogenized and 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.

Characterization of nonextractable residues: Egg yolk and tissue nonextractables were incubated overnight with protease from Streptomyces griseus (Sigma Chemicals) at 37 C. The samples were then filtered or centrifuged and the soluble residues concentrated. The solution was washed with hexane and partitioned with butanol followed by ethyl acetate. The organic phases were combined, concentrated, and analyzed by TLC. The aqueous phases were cleaned up by C18 chromatography, DEAE Sephadex chromatography, and characterized by TLC analysis. Aliquots of both the organic and aqueous phases were subjected to beta-glucuronidase catalyzed hydrolysis and analyzed by DEAE Sephadex chromatography.

Structural assignments for metabolites were made from comparison of migration on TLC to that of standard compounds. Structures were confirmed using GLC, HPLC, GLC/MS, or LC/MS methods.

Approximately 80% of the ^{14}C-activity administered to the hens was recovered from excreta (79%), egg yolks (<0.01%), egg whites (0.07%) and tissue samples (1%). The radioactivity in eggs reached a plateau on about the fourth day.

The characterization of residues in tissues, eggs and excreta is shown in Table 1. Radioactive residues in egg whites and excreta were soluble in methanol:water (9:1); however, nonextractable residues comprised 24-69% of the total radioactive residues (TRR) in egg yolks and tissues. Treatment of these matrices with a protease prior to methanol:water (9:1) extraction reduced nonextractables to 0-18% of the TRR. The registrant concluded that these residues were occluded in tissues and were released as a result of protease treatment. An analysis of protease-released residues from liver is shown in Table 2.

The combined residues of G-27550 (M1), GS-31144 (M2) and 2-(beta-hydroxy isopropyl)-6-methyl-4(1H)-pyrimidinone (M3) comprised 10% of the TRR from liver. Protease treatment of the tissue before extraction raised this total to 56% of the TRR. Attempts to purify residues from other tissues and egg yolks after protease treatment were not successful.

Table 1. Partial characterization of soluble ^{14}C-residues in tissues, eggs, and excreta of laying hens following oral dosing for 7 days with [ring-^{14}C]diazinon.

^{14}C-Residues[1]	Percent of TRR in sample							
	Egg yolks	Egg whites	Liver	Kidney	Lean meat	Skin	Peritoneal fat	Excreta
Extractable[2]	67/88	98/-	63/82	76/98	64/94	44/100	31/100	97/-
Organic sol.	88	87	49	48	49	61	62	74
Aqueous sol.	12	13	51	52	51	39	38	26
Nonextractable	33/12	2/-	37/18	24/2	36/6	56/0	69/0	3/-
Organic Extractables								
[B1] Diazinon	0.02	0.03	0.03	0.08	0.04	0.89	2.00	14.93
[B2] CGA-14128	0.06	0.05	<0.01	0.11	0.03	0.02	1.37	1.73
[B3] G-24576[3]	0.42	1.28	0.89	0.18	0.24	1.25	0.77	1.29
[BX] Unknown[3]							1.00	
[BY] Unknown[4]						6.30		
[M1] G-27550[4]	11.14	9.38	0.59	2.26	1.98	2.61	0.73	5.96
[MX] Unknown[4]	2.89							0.93
[M2] GS-31144	18.57	33.34	3.46	3.69	6.52	4.16	3.06	10.83
[M3] 2-(Beta-hydroxy isopropyl)-6-methyl-4(1H)-pyrimidinone			1.98	5.73		2.31		7.18
[G1/G2/G3/others] Glucuronide conjugates and/or others	25.18[5]	41.27[5]	23.46	24.59	22.39[5]	9.69	9.71[5]	27.99
Aqueous Extractables								
[B1/B2/B3/M1/M2]	2.57	1.45	5.01	0.36	0.17	0.63	3.67	0.66
[M3]	2.22	8.78	4.02	7.51	1.50	8.24	4.64	5.07
[G1]	0.76	1.47	2.14	7.90	15.37	4.31	1.07	1.51
[G2]	1.02	0.61	4.99	12.97	7.84	2.23	1.62	6.89
[G3/Others]	1.47	0.45	15.98	10.83	7.51	1.77	0.78	11.02

[1] Symbols in brackets indicate TLC zones.
[2] Values before slash indicate solubility before treatment of tissue with protease. Values after slash indicate solubility after protease treatment.
[3] Registrant proposes this is unresolved CGA-14128
[4] Registrant proposes this is unresolved GS-31144 and G-27550.
[5] Probably unresolved [M3] and [G1/G2/G3/others].

Table 2. Comparative TLC analysis of metabolites in liver extract before and after proteolysis treatment[1]

TLC Zone	% ^{14}C in Liver: Liver Extract Before Protease	Liver Extract After Protease
B1	0.05	0.24
B2	<0.01	0.56
B3	1.42	2.08
M1	0.93	31.68
M2	5.49	13.68
M3	3.14	10.64
G1/G2/G3/others	37.24	25.52

[1] See Table 1 for metabolite identities for TLC zones.

In summary, total radioactive residues in tissues ranged from 0.010 ppm in peritoneal fat to 0.149 ppm in kidney. Approximately 31-76% of the residues in tissues were characterized. Approximately 7-16% of TRR in tissues was characterized as diazinon, CGA-14128, G-24576, G-27550 and GS-31144; approximately 18-70% was characterized as 2-(beta-hydroxy-isopropyl)-6-methyl-4(1H)-pyrimidinone (M3), glucuronide conjugates and other conjugates. Approximately 98% of the TRR in egg yolks and 66% of the TRR in egg whites were characterized. The major metabolites in egg yolks and egg whites were GS-31144 (19% and 33%), G-27550 (11% and 9%), and smaller amounts of diazinon, CGA-14128 and G-24576. Metabolite M3, glucuronide conjugates and/or other conjugates comprises ca. 31% and 53% of the TRR in egg yolks and egg whites, respectively. The registrant proposes the same metabolic pathway in laying hens as in goats; the dephosphorylation of diazinon to corresponding pyrimidinols and oxidation at the isopropyl side chain to produce hydroxy metabolites. These metabolites can undergo conjugation before excretion from the animal.

cc: W.O. Smith, Diazinon Registration Standard File, Diazinon Subject File, RF, Circ. (7), J. Burrell (FOD)

H7509C:DEB:X4353:CM#2:RM812D:W.O.Smith:ws:2/14/90