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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **Diflubenzuron. Issues Presented at the 3/17/94 Meeting of the HED Metabolism Committee.** Estimates of Residue Levels of Diflubenzuron and p-Chloroaniline to be Used for Dietary Risk Calculations and Estimate of Dietary Risk. Reregistration Case No. 0144. Chemical No. 108201. No MRID #. No DP Barcode. No CBRS #.

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TO: HED Metabolism Committee

Tolerances for residues of the insecticide diflubenzuron (DFB, N-[[4-chlorophenyl)amino] carbonyl]-2,6-difluorobenzamide) are established in various racs (40 CFR 180.377) and feed additives (40 CFR 186.2000).

Metabolism studies indicate that in animals and mushrooms, DFB metabolites include p-chloroaniline (PCA), chlorophenylurea (CPU), p-chloroacetaniline (PCAA), and other moieties.

Conclusions

A risk analysis using a Q_1^* for PCA of 0.059, as provided by Dr. Engler, and residue values for DFB and PCA (and related compounds calculated as PCA) was performed. Risk was calculated based on existing DFB uses and the pending citrus use. Risk was also calculated both with and without 2% in vivo conversion of DFB to PCA. Results are presented in Table 1.

Table 1. Summary of risk calculations for DFB and PCA (and related compounds).

Risk Scenario		Low Animal Dietary Burden	High Animal Dietary Burden
Current Tolerances Only	No in vivo conversion of DFB to PCA	9.8×10^{-7}	1.5×10^{-6}
	With 2% in vivo conversion of DFB to PCA	9.89×10^{-7}	1.52×10^{-6}
Current Tolerances and Pending Citrus Tolerance	With 2% in vivo conversion of DFB to PCA	1.13×10^{-6}	1.66×10^{-6}

* Low animal dietary burden assumes 5% of cattle receive DFB bolus use (and use is evenly distributed across the US), high dietary animal burden assumes 100% of cattle receive DFB bolus (contributes 4.0 ppm to animal dietary burden).

NOTE: The majority of the risk (approximately 80% of total) is attributable to PCA and related compounds found in mushrooms. BEAD at this time is unable to provide estimates for the percent of mushrooms treated with DFB, therefore 100% crop treated was assumed. Data from a mushroom metabolism study were used to estimate levels of PCA and related compounds (see Detailed Considerations below). **Both the DFB Registration Standard (11/16/84) and the Registration Standard Update (7/21/91) required mushroom magnitude of the residue studies. Data from these studies, in conjunction with percent crop treated data, should be used to refine the risk estimate when producing the DFB RED.**

Detailed Considerations

Methods

1. Determination of DFB Levels in RACs
   a. Plants - Levels of DFB in plants were obtained by multiplying tolerances by percent crop treated.
   b. Animals - Levels of DFB in animal tissues were determined using information provided in metabolism studies, or assuming that the portion of the TRR not consisting of PCA (and related compounds) was DFB.
2. Determination of PCA and Related Compounds - CBRS used results from metabolism studies to determine the percent of TRR present as PCA or related compounds (CPU and PCAA). Then, using tolerance levels for diflubenzuron, and adjusting for percent crop treated, total levels of PCA and related compounds were estimated.
3. In vivo conversion of DFB to PCA - Based on a recommendation from TOX (H.Spencer, 3/21/94), 2% in vivo conversion of DFB to PCA was assumed.

Collected 3/23/94
SK
3/24/94

Results for levels of DFB and PCA (and related compounds) are summarized in Tables 2.a. and 2.b.

Table 2.a. **Fungi and Plants** - Levels of DFB or PCA and related compounds (CPU and PCAA) used in risk assessment.

Commodity	Tolerance (ppm)	Percent Crop Treated	Amount DFB to be used for Risk Assessment (ppm)[a]	Amount PCA to be used for Risk Assessment (ppm)
Mushrooms	0.2	100 (Unknown)	0.2	0.69
Soybeans	0.05	<1	0.0005	--
Walnuts	0.05	100	0.05	--
Cottonseed	0.2	<10	0.02	--
Citrus, pulp[b] juice[b] peel[c]		100	0.05 0.05 0.5	-- -- --

[a] This number was multiplied by 2% to account for in vivo conversion of DFB to PCA.
[b] From memo by S.Malak, 2/12/94.
[c] Based on pending tolerance for whole fruit.

Table 2.b. **Animals** - Levels of DFB or PCA and related compounds (CPU and PCAA) used in risk assessment.

Commodity	Tolerance (ppm)	Amount DFB to be used for Risk Assessment (ppm)[a]		Amount PCA to be used for Risk Assessment (ppm)	
		High Dietary Burden[b]	Low Dietary Burden[b]	High Dietary Burden	Low Dietary Burden
Milk	0.05	0.0018	0.0003	0.0022	0.0004
Eggs	0.05	0.00018	--	0.0004	--
Fat (cattle, goat, hogs, horses, sheep)	0.05	0.002	0.0003	--	--
Mbyp (cattle, goat, hogs, horses, sheep)	0.05	0.111	0.0173	0.0237	0.0037
Meat (cattle, goat, hogs, horses, sheep)	0.05	0.0005	0.00007	--	--
Poultry, fat	0.05	0.00018	--	--	
Poultry, mbyp	0.05	0.00003	--	0.00006	--
Poultry, meat	0.05	0.00004	--	0.000012	--

[a] This number was multiplied by 2% to account for in vivo conversion of DFB to PCA.
[b] For cattle, high dietary burden represents 4.5 ppm DFB (includes bolus use) and low dietary burden includes bolus use with 5% of cattle treated. Dietary burden for poultry is 0.002 ppm.

Metabolism Studies

Fungi

Mushrooms - A review of the metabolism study "Residues in Mushrooms grown from compost treated with radiolabeled diflubenzuron", dated 2/86, was completed by M. Nelson (4/22/87, CBTS #2085, Acces. No. 265703). In this study, mushroom compost, consisting mostly of horse and chicken manure, was spawned with mushroom culture and then was treated (day 1) with diflubenzuron, either ^{14}C-labeled in the 4-chlorophenyl ring of ^{3}H-labeled in the 2,6-difluorobenzoyl moiety. The dosage rate was 1 gm ai/m^2 (assuming a bed depth of 5 inches, this rate corresponds to 22 gm ai/m^3, or 0.05 lb ai/100 ft^3, which represents 0.5X the maximum application rate). An additional application was made at casing (day 13). Samples of mushrooms were collected at each of the first four flushes (day 32, 38, 46, and 55). Each flush was analyzed separately. TRR was determined by combustion analysis. Quantitation of diflubenzuron (DFB) and its metabolites CPU, DFBA, and PCA was via reversed phase isotope dilution, and TLC with autoradiography using reference compounds.

Residues of diflubenzuron (DFB), 4-chlorophenylurea (CPU), diflubenzoic acid (DFBA), and 4-chloroaniline (p-chloroaniline, PCA) were detected at levels up to 0.18, 0.6, 3.96, and 0.02 ppm respectively.. No questions were raised concerning this study because the data gap which this study was submitted to fulfill had been previously considered fulfilled in a S.Malak memo of 7/28/86.

The tolerance for DFB in/on mushrooms is 0.2 ppm. If DFB were present in/on mushrooms at the tolerance level, combined residues of CPU and PCA could be present at 0.69 ppm.

Plants

Citrus - A CBTS review of a citrus metabolism study (S. Malak, 7/27/89, CBTS #5426-5429, in PP#F2507, MRID #41079301) noted several deficiencies, which were subsequently resolved (S.Malak, 10/2/89; CBRS #5706 and 5707; S.Malak, 11/21/89, CBRS #5913, 5978-5983). The reviews concluded that the vast majority of the residue in citrus fruit is composed of unchanged parent compound, and no detectable levels (<1 ppb) of PCA, CPU, or DFBA were present.

No tolerances for DFB in/on citrus have been established. Results of the citrus metabolism study were presented only to demonstrate that PCA, CPU, and PCAA were not found at levels >1 ppb.

Apples - An apple metabolism study has been previously submitted and reviewed in conjunction Diflubenzuron Registration Standard (11/16/84). By use of reverse phase isotope dilution analysis, PCA was detected in apple leaves and fruit at levels of 1.15 and 0.002 ppm respectively. Duphar contended that PCA found in leaves and fruit was an artifact that could have resulted due to extraction techniques in boiling methanol/water (1:1) (S.Malak, 2/25/88, PP#5F3270/FAP#5H5472), however, CBRS was unable to conclude if PCA found was actually a metabolite, or an artifact, or an impurity in the test substance (S.Malak, 8/24/88). Duphar submitted a new apple metabolism study which was reviewed by CBTS (J.Stokes, 5/27/93, CBTS #9703, MRID #42127700 and 42127701). This study was not adequate because: 1) low recoveries for PCA; 2) the application rate was too low; and 3) a PHI of 63 days instead of the proposed label PHI of 28 days was used. A new apple metabolism study was requested.

No tolerances for DFB in/on apples have been established. CBRS concludes that the presence of PCA as a DFB metabolite in apples remains unresolved.

Soybeans - Tolerances for residues of diflubenzuron in/on soybeans are established 0.05 ppm, and feed additive tolerances for residues of DFB in/on soybean hulls and soapstock have been established at 0.5 and 0.1 ppm respectively (40 CFR 186.2000). A recently submitted soybean metabolism study is currently under review (MRID #4265801, CBRS #11,501). Based on preliminary review of this study, the >90% of the TRR in soybean leaves was unchanged parent (ppm levels not provided). DFBA, CPU, and PCA were not detected, but the limit of detection for these compounds was not provided. In soybean hulls, 81.4% to 97.9% of the TRR (6.57 -17.5 ppm) was identified as unchanged parent. Again, DFBA, CPU, and PCA were not detected. The limit of detection for these compounds was 0.3 ppm. Residues in soybean seeds were too low to allow for metabolite characterization (<0.1 to 0.038 ppm).

Pending final review of the soybean metabolism study, CBRS tentatively concludes that PCA, CPU, and PCAA are not present in soybeans at levels ≥0.3 ppm.

Animals

Livestock Dietary Burden Calculations

DFB has tolerances with regional registrations for use on pasture and range grass. Use on pasture grass is restricted to irrigated pastures in central CA for control of mosquitos. This pasture grass is to be used only for grazing; it is not to be cut for hay or silage. Use on range grass is restricted to CO, NM, OK, and TX for control of range caterpillars.

BEAD estimated that <10% of pasture in CA is treated with DFB. Additional information supplied by BEAD indicates that <1% of range would be treated with DFB. BEAD stated that <1% of all rangeland is treated with any insecticide, and DFB is a "small player" in this area, so the estimate of percent crop treated is conservative.

DFB is also registered for use as a controlled release bolus in beef and dairy cattle for the control of flies in manure. This use has been calculated to contribute 4.00 ppm to the dietary intake (S.Malak, 2/20/91, CBTS #6529-6534). BEAD estimated that <5% of cattle are administered DFB as a bolus.

Data used to provide estimates of livestock dietary burdens of DFB are found in Table 3.

Table 3. Data used to provide estimates of livestock dietary burdens of DFB.

Feed Item	Tolerance (ppm)	Percent Dry Matter	Percent Crop Treated	Percent in Feed			Maximum Dietary Intake (ppm)		
				Beef Cattle	Dairy cattle	Poultry	Beef Cattle	Dairy cattle	Poultry
Grass, rangeland, forage	3.0	25	<1	60	70	NU (not used)	0.072	0.084	--
Cottonseed	0.2	88	<10	25	25	NU	0.006	0.006	--
Soybean seed	0.05	89	<1	15	20	20	0.0001	0.0001	0.0001
Soybean Hulls	0.5	90	<1	20	20	20	0.0011	0.0011	0.0011
Soybean Soapstock	0.1	99	<1	5	5	5	0.00005	0.00005	0.00005
Orange pulp, dehydrated[a]	1.0	91	100	33	33	NU	0.363	0.363	--
Bolus, controlled release[b] (assuming 100% crop treated)	--						4.00	4.00	--
Bolus, controlled release[b] (assuming 5% crop treated)	--						0.2	0.2	
TOTAL			with 100% crop treated Bolus use				4.44	4.45	0.00125
			with 5% crop treated Bolus use				0.642	0.654	

[a] Pending tolerance.
[b] BEAD estimates that <5% of cattle are treated with controlled release DFB bolus.

<u>Ruminant Metabolism</u> - CBTS has reviewed a goat metabolism study submitted by Duphar (J.Stokes, 12/30/92, CBTS #10,828, and J.Stokes 2/23/93, CBTS #11,183). In this study, four female lactating goats were orally dosed twice a day with ^{14}C-DFB at 0.1 or 2.4 mg/Kg body weight. The low dose rate represents approximately 10 ppm and the high dose 250 ppm based on feed consumption of 1 Kg/day.

Uptake of ^{14}C was low by all tissues. Of the administered ^{14}C dose, 77% to 81% was excreted in the feces, 8% to 15% was found in the intestinal tract contents, and 6% to 12 % was excreted in the urine. The total radioactivity recovered in the feces, intestinal tract contents and urine of the was 97% and 102% respectively for the low and high dose goats. TRR levels for various tissues are presented below in Table 4.

Identification of residues in liver and milk was made using HPLC and GC techniques. Levels of compounds of interest to the Metabolism Committee are shown in Table 5.

Table 4. TRR (^{14}C-DFB equivalents in organs, tissues and body fluid of goats. Goats were dosed with ^{14}C-DFB at 0.1 or 2.5 mg/Kg/body weight/day twice daily for 3 consecutive days. Based on an average feed consumption of 1 kg/day, the low dose represents 10 ppm in the feed and the high dose 250 ppm. TRR was determined by combustion analysis.

Sample	10 ppm Dose		250 ppm Dose	
	Goat 1 (ppm)	Goat 3 (ppm)	Goat 4 (ppm)	Goat 5 (ppm)
Milk	0.009	0.007	0.22	0.22
Liver	0.217	0.262	3.24	6.06
Kidney	0.019	0.016	0.36	1.02
Spleen	0.002	0.002	0.05	0.07
Fat	0.003	0.004	0.12	0.30
Muscle	ND	0.001	0.02	0.05
Bile	0.151	0.229	20.75	2.59
Intestines (contents/walls)	0.214	0.187	4.41	9.53
Carcass	0.006	0.008	0.12	0.18

Table 5. PCA and CPU levels found in Liver and Milk of Goats. Goats were dosed with ^{14}C-DFB at 0.1 or 2.5 mg/Kg/body weight/day twice daily for 3 consecutive days. Based on an average feed consumption of 1 kg/day, the low dose represents 10 ppm in the feed and the high dose 250 ppm.

Sample	Metabolite	10 ppm Dosing Level				250 ppm Dosing Level			
		Goat 1		Goat 3		Goat 4		Goat 5	
		% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Liver	PCA	--	--	--	--	0.34	0.011	0.46	0.028
	CPU	16.5	0.036	11.3	0.030	12.8	0.415	15.0	0.909
Milk	CPU	41.8	0.004	28.6	0.002	32.5	0.072	54.5	0.120

Estimate of DFB and PCA Related Compounds present in Ruminant Tissues

Table 6 provides estimates of PCA and related compounds possibly present in ruminant liver and milk based on ruminant metabolism study and the above calculated anticipated dietary burdens. Table 7 presents estimates of levels of DFB found in ruminant tissues.

Table 6. Estimates of PCA and related compounds possibly present based on ruminant metabolism study and anticipated dietary burden.

Matrix	Residue Level Found in Metabolism Study at 10 ppm Dosing Level (See Table 3)	Calculated Residue Level with Dietary Burden of:		% PCA Related Compounds Found in Metabolism Study (see Table 4)	Calculated Levels of PCA Related Compounds (ppm)	
		4.5 ppm	0.7 ppm		4.5 ppm Diet Burden	0.7 ppm Diet Burden
Liver	0.3 ppm	0.135 ppm	0.021 ppm	0.5% (PCA)	0.0007 ppm	0.0001 ppm
				17% (CPU)	0.0230 ppm	0.0036 ppm
Milk	0.009 ppm	0.004 ppm	0.0007	55% (CPU)	0.0022 ppm	0.0004 ppm

Table 7. Estimates of DFB based on ruminant metabolism study and anticipated dietary burden.

Matrix	Calculated DFB Level With Animal Dietary Burden of:	
	4.5 ppm	0.7 ppm
Liver	0.111 ppm	0.0173 ppm
Milk	0.0018	0.0003
Kidney	0.009	0.002
Fat	0.002	0.0003
Muscle	0.0005	0.00007

Poultry - A poultry metabolism study (MRID #00070186), submitted by Thompson Hayward Chemical Co., was discussed in the Diflubenzuron Registration Standard. In this study, laying hens were dosed with uniformly double ring labeled ^{14}C-diflubenzuron at 0.05, 0.5, and 5 ppm in the diet for 1-28 days. At all dosing levels, ^{14}C residues plateaued in tissues and eggs by day 10 of dosing. TRR levels for tissues are presented in Table 8.

Table 8. TRR levels (ppm) in hens dosed with DFB at 0.05, 0.5, and 5 ppm in the feed for 1 to 28 consecutive days.

Tissue	Dosing level (ppm)		
	0.05	0.5	5
Fat	<0.0006 - 0.0067 ppm	<0.005 - 0.033 ppm	0.078 - 1.16 ppm
Kidney	<0.0006 - 0.0026 ppm	<0.005 - 0.013 ppm	0.068 - 0.338 ppm
Liver	<0.0006 - 0.0026 ppm	<0.005 - 0.044 ppm	0.059 - 0.453 ppm
Muscle	<0.0006 - 0.0017 ppm	<0.005 ppm	<0.032 - 0.099 ppm
Eggs	<0.0006 - 0.0029 ppm	<0.005 - 0.100 ppm	<0.032 - 0.833 ppm

Tissues and eggs collected after 7 days of continuous dosing at 5 ppm were extracted with ethyl acetate and residues were characterized by TLC. Of the total ^{14}C residues present in tissues and eggs, the percent TRR of DFB, DFBA and CPU present are shown in Table 9. Small amounts of PCA were tentatively identified in some samples.

Table 9. Identification of radioactive residues in hens dosed with DFB for 7 days at 5 ppm in the feed. Results are expressed as percent of TRR (see Table 5 for ppm values).

Tissue	% DFB	% DFBA	% CPU
Fat	100	0	0
Muscle	63.4 - 66.3	6.8 - 9.2	13 - 22.1
Liver	18.6	7.4	49.8
Kidney	23.8	0	40
Eggs	68.8	3.7	11.2

Extrapolation from the 5 ppm feeding level to the anticipated dietary burden of 0.0013 ppm, and multiplying the result by the percent of TRR identified as DFB and CPU yields results shown in Table 10.

Table 10. Levels of DFB and PCA in Poultry tissues based on poultry metabolism study and dietary burden of 0.0013 ppm.

Poultry Matrix	DFB (ppm)	PCA and Related Compounds (ppm)
Fat	---	0.00018
Muscle	0.000012	0.00004
Liver	0.00006	0.00003
Kidney	0.00004	0.00003
Eggs	0.00004	0.00018

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