Abbotts 96-23

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

MAY 14 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Atrazine (080803), Reregistration Case No. 0062 and Special Review. Registrant Ciba-Geigy Corporation. Residues in Cow Milk.
CBRS No. 16986, DPBarcode No. D223985, MRID 43934401.

FROM: John Abbotts, Chemist
Special Review Section I
Chemistry Branch II - Reregistration Support
Health Effects Division [7509C]

THRU: Andrew R. Rathman, Section Head
Special Review Section I
Chemistry Branch II - Reregistration Support
Health Effects Division [7509C]

TO: Kathryn Boyle
Special Review Section
Risk Characterization and Analysis Branch
Health Effects Division [7509C]

and Joseph Bailey
Special Review Branch
Special Review and Reregistration Division [7508W]

Registrant Ciba-Geigy Corporation submitted data on transfer and nature of the residues in milk when atrazine is fed to cows. Assignment instructions are to review the submission. Conclusions and Recommendations below pertain only to data in the present submission.

Tolerances are established for residues of the herbicide atrazine, 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine, in or on agricultural commodities (40 CFR 180.220(a)), and for combined residues of atrazine and its metabolites 2-amino-4-chloro-6-ethylamino-*s*-triazine (G-28279), 2-amino-4-chloro-6-isopropylamino-*s*-triazine (G-30033), and 2-chloro-4,6-diamino-*s*-triazine (G-28273), in or on specified plant commodities (40 CFR 180.220(b)); see Figure 1 for structures. Atrazine is a List A Chemical. The Residue Chemistry Chapter was issued 7/25/83; the Registration Standard (Guidance Document) was issued 9/85; a Second Round Review (SRR)

Residue Chemistry Chapter was issued 10/18/88. Special Review has been initiated on triazine herbicides, including atrazine (59 FR 60412, 11/23/94, PD1).

Conclusions

1. The present submission was an unsolicited study from the Registrant; it was not submitted in response to any Agency DCI.

2. The chloro metabolite G-28273 (see Figure 1) was the most prominent residue in milk samples, consistently representing 60-70% of TRR; no other metabolite represented more than 4% TRR in milk.

3. The transfer ratios for residues in milk:atrazine residues in feed from the present submission at doses below 1 ppm are reasonably consistent with ratios from previous studies at higher doses, for both TRR and chloro metabolites.

Recommendations

Data from the present submission should be used in exposure assessment for milk where anticipated residues of chloro metabolites in feed are less than 1 ppm. The data provide some confidence that it is reasonable to use available data from cattle metabolism/feeding studies in exposure assessment for other cattle commodities.

We recommend that the Registrant be provided a copy of this memo.

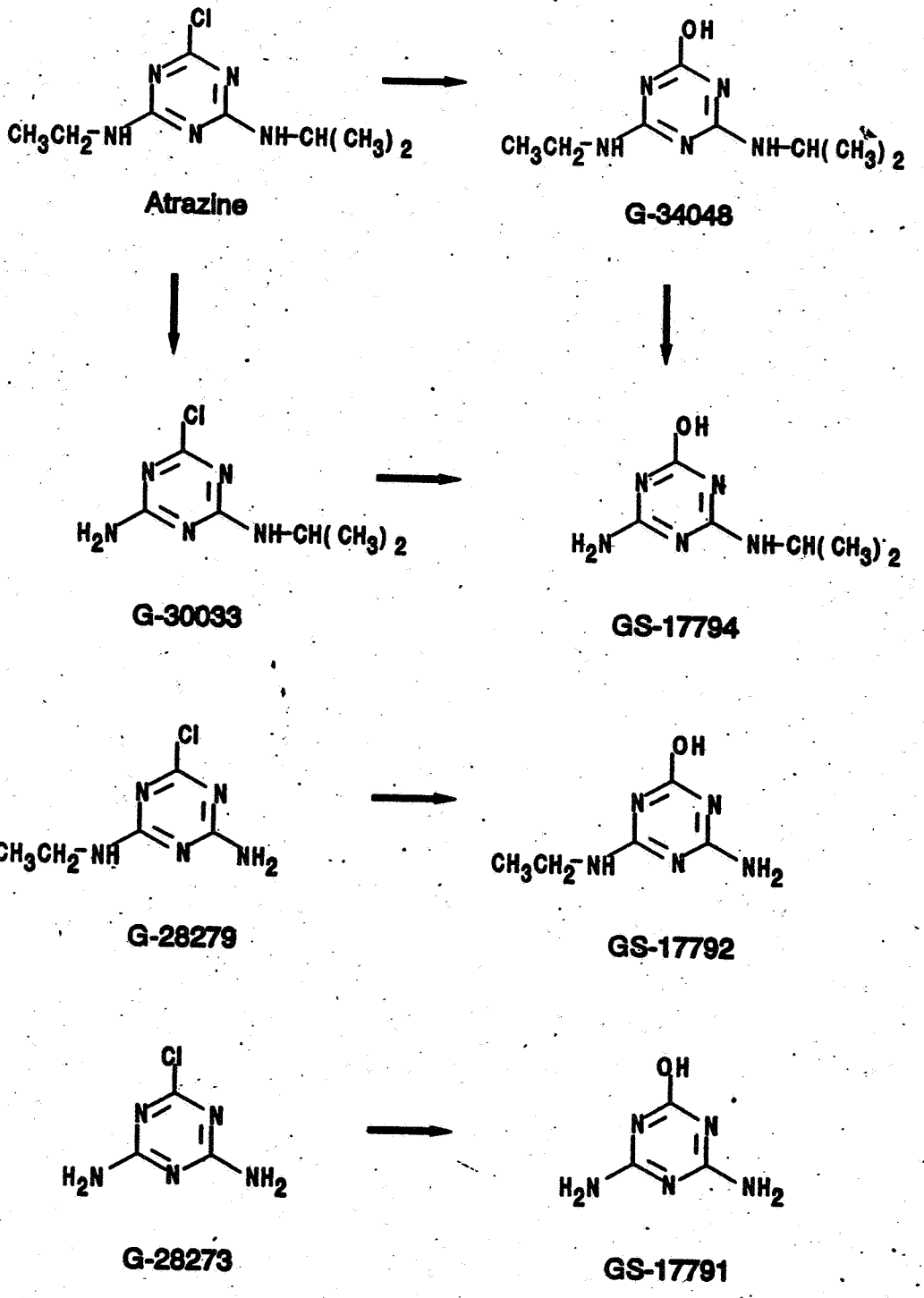

Figure 1. Atrazine, chloro (left), and hydroxy metabolites

DETAILED CONSIDERATIONS

Present Submission

The Registrant submitted the following document:

Determination of Transfer Rate and Nature of the Residue(s) in Milk from ^{14}C-Atrazine Treated Cows, Corning Hazleton, Inc, Madison WI, Project CHW 6117-325, Sponsor Ciba-Geigy Corporation, January 16, 1996 (MRID 43934412).

We note at the outset that this study was not submitted to meet the requirements of any Agency DCI:

Conclusion 1: The present submission was an unsolicited study from the Registrant; it was not submitted in response to any Agency DCI.

The abstract of the present submission notes that this study was conducted to determine the rate of transfer and the nature of the residues present in milk. Lactating dairy cattle were dosed with atrazine at levels of 0.764 ppm or lower in the feed, dry weight basis. This study provides data additional to cattle feeding/metabolism data previously provided to the Agency, based on feeding levels of 3.8 to 38 ppm atrazine.

The performing laboratory for animal handling and radiochemical analysis was Corning Hazleton, Madison WI. The test substance was [^{14}C]-atrazine, uniformly labeled in the triazine ring. Radiochemical purity of the test substance was 98%. Specific activity of the test substance was 50.8 μCi/mg.

The test substance was placed in capsules and administered by balling gun to lactating Jersey cows daily within 30 min after the morning milking, for nine consecutive days. Four cows were used in the study, at daily doses in the feed of 0.0085, 0.0747, and 0.764 ppm atrazine, respectively; one cow was the control animal. Cows were milked twice daily, in the morning and afternoon, and were observed for general health at the same times. Animals were sacrificed within 8 h of the final milking, but tissues were not analyzed. Milk samples were stored refrigerated for up to one week, then stored frozen at -10°C or colder until analysis. Initial chromatographic analysis was performed within three months of collection for all samples, and in some cases within a week of collection. A single urine sample was collected from the high dose cow on day 6 of dosing, stored refrigerated for 4 days, then stored frozen.

Milk samples were homogenized by shaking, then mixed with scintillation cocktail and total radioactive residues (TRR) were determined directly by liquid scintillation counting (LSC).

Liquid fractions from the extraction process were also analyzed directly by LSC. Solid residues remaining during the extraction process were analyzed by combustion and LSC.

Total radioactive residues in milk reached a plateau on day 3, and remained at similar levels through day 9. Residue levels were always lower from the morning milking, 24 h after dose administration, than from the afternoon milking 10 h after dose. Table 1 summarizes average TRR levels in milk from 10 h, 24 h, and composite samples:

Table 1. Total radioactive residues in milk.

Dose level, ppb atrazine	TRR, ppb, at sample time after daily dose:		
	10 hours	24 hours	Composite
8.5 (Low)	0.214	0.108	0.161
74.7 (Middle)	1.59	0.817	1.19
764 (High)	16.1	8.0	11.7

Table notes: TRR values are averages for days 3 through 9, when levels for each dose reached a plateau.

Extraction

The most extensive extraction protocol was Method II, outlined in Figure 2. This method, used with samples from the low dose cow, employed an additional extraction step for improved sample cleanup. Method I, which omitted the additional step, was used with samples from each dose level. Milk was mixed with twice the volume of acetone and stirred for 30 min. Solutions were centrifuged and the supernatant was decanted. The remaining fraction was extracted a second time with acetone, and supernatants were pooled and filtered. Acetone was removed by rotary evaporation, and the resulting solution was partitioned three times with hexane to produce aqueous and hexane fractions, which were each concentrated by rotary evaporation. Extraction Method I ended at this step.

With Extraction Method II, the aqueous layer following hexane partitioning was partitioned three times with ethyl acetate. Ethyl acetate layers were pooled, and the ethyl acetate and aqueous extracts were each concentrated by rotary evaporation. Table 2 summarizes the distribution of radioactivity in cow milk during extraction:

Table 2. Distribution of TRR in milk extracts.

Fraction	% TRR in fraction by dose level for:			
	Low, Method II	Low, Method I	Middle	High
Hexane	0.9	2.1	1.7	0.6
Aqueous	17.2	82.2	90.3	86.0
Ethyl Acetate	72.9			
Unextracted	19.6	19.5	18.7	19.0

Table notes: Values are averages for samples from days 1 through 9 for Method I, and averages for samples from days 5 through 9 for Method II. Extraction protocols are outlined in Figure 2; ethyl acetate extraction was only used with Method II (Low dose).
Actual dose levels and average TRRs are indicated in Table 1.

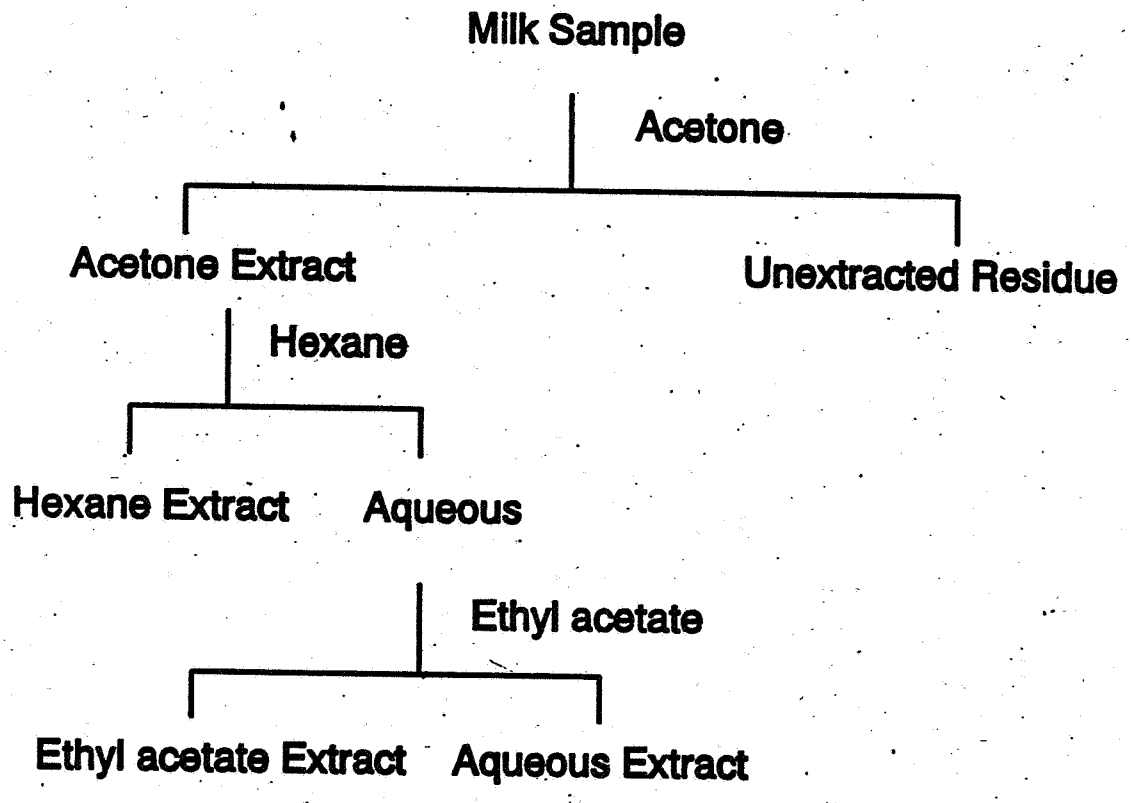

Figure 2. Extraction Method II for milk.

Residue Analysis

Selected fractions were analyzed by thin layer chromatography (TLC), HPLC, or cation exchange chromatography. For TLC, silica gel plates were developed in 100% ethyl acetate. Reverse phase HPLC analysis was conducted using a YMC-Pack AQ-303 column. This column was eluted with gradients in 1% acetic acid in water as Solvent A, and 1% acetic acid in acetonitrile as Solvent B. Cation exchange chromatography was with an Aminex A-4 column, eluted with a linear ammonium formate gradient from 0.1 M to 1.0 M, followed by 0.1 M ammonium hydroxide. Residues were assigned based on chromatographic behavior of standards, which were the chloro and hydroxy compounds shown in Figure 1.

The only metabolite representing a significant portion of TRR in milk samples was G-28273 (Figure 1), which was identified by TLC, HPLC, and/or Aminex chromatography; no other metabolite represented more than 4% TRR in milk. HPLC analysis of the urine sample also indicated that G-28273 was the most prominent metabolite.

The performing laboratory determined levels of G-28273 in milk for each composite sample from each day for the middle and high dose cows. For these samples, levels of G-28273 were determined by HPLC for each day, and also by TLC on day 5, and TLC and Aminex chromatography on day 9. For the low dose samples, levels of G-28273 were determined by TLC and Aminex on days 5 and 9. The data presented lead to the following comment:

Conclusion 2: The chloro metabolite G-28273 (see Figure 1) was the most prominent residue in milk samples, consistently representing 60-70% of TRR; no other metabolite represented more than 4% TRR in milk.

Milk samples were stored frozen for less than 6 months. Supporting storage stability data therefore were not required.

The Registrant concluded that over the three dose levels examined, there was a linear relationship between atrazine dose in the feed and residues of G-28273 in milk. Table 3 summarizes transfer ratios for TRR and G-28273, using average residues during the plateau period of days 3 through 9:

Table 3. Transfer ratios in cow milk.

Atrazine dose, ppb	Residues, ppb [transfer ratio], for:	
	TRR	G-28273
8.6	0.161 [0.019]	0.11 [0.013]
74.7	1.19 [0.016]	0.80 [0.011]
753	11.7 [0.016]	8.27 [0.011]

Table notes: Residues are averages during days 3-9, when residues reached plateaus.
Transfer ratios are residues in milk:atrazine residues in feed.

Comparison with Previous Data

Chemistry Branch has previously determined anticipated residues for atrazine, including anticipated residues in animal commodities, using metabolism and/or feeding data then available. Anticipated residues were initially determined for atrazine and chloro metabolites (DEB 3688-3703, 3756, 9/14/88, M.S. Metzger). With this determination, anticipated residues in cattle commodities were based on feeding studies in which cattle were fed 3.75, 11.25, or 37.5 ppm atrazine in the diet. The only residue detected in milk was G-28273; atrazine and other chloro metabolites were not detected. Maximum levels of G-28273 at each feeding level were 0.03, 0.12, and 0.46 ppm, respectively. On the basis of these data and anticipated residues in dairy cattle feed of 0.784 ppm, anticipated residues in milk of 0.004 ppm were determined (Ibid.). This indicates a transfer ratio of 0.005 to calculate chloro residues in milk:atrazine residues in feed.

The Second Round Review Residue Chemistry Chapter (10/18/88) reported a conclusion for metabolism in cow that the total terminal residue in milk included 3% atrazine, 2.5% G-30033, 5% G-28279, and 69.1% G-28273. No free or bound hydroxy metabolites were detected. Based on hydrolysis of residues to cyanuric acid, total triazine residues accounted for 86% TRR. These data were based on metabolism studies with cattle fed labeled atrazine at 0.62 ppm, 6.8 ppm, and 28 ppm in the daily diet for 10 days. Maximum TRR in milk were 0.01, 0.13, and 0.67 ppm, respectively. Applying data on nature of the residues to the highest TRR gives a transfer ratio of 0.019 for chloro residues in milk.

Anticipated residues were subsequently revised by assuming that total radioactive residues represented total triazine ring residues (DEB 5783, 5/3/90, M.S. Metzger). That revision reported data from a variety of metabolism studies. Data on cattle milk are summarized in Table 4, and transfer ratios have been calculated:

Table 4. Milk transfer ratios from previous studies with cattle.

Dose in feed, ppm	Total radioactive residues, ppm [Ratio, residues in milk:residues in feed]
28	0.67 [0.024]
6.8	0.12 [0.018]
0.62	0.01 [0.016]

Table notes: Data are taken from DEB 5783, 5/3/90, M.S. Metzger. Transfer ratios have been calculated here. Radiolabel was ^{14}C-atrazine in all cases.

The data from the present submission show a good degree of consistency with data from previous studies for transfer to milk. These considerations lead to the following comments:

Conclusion 3: The transfer ratios for residues in milk:atrazine residues in feed from the present submission at doses below 1 ppm are reasonably consistent with ratios from previous studies at higher doses, for both TRR and chloro metabolites.

Recommendations: Data from the present submission should be used in exposure assessment for milk where anticipated residues of chloro metabolites in feed are less than 1 ppm. The data provide some confidence that it is reasonable to use available data from cattle metabolism/feeding studies in exposure assessment for other cattle commodities.

cc:Circ, Abbotts, RF, Atrazine List A File, Atrazine SF
RDI:ARRathman:5/6/96:RBPerfetti:5/13/96:EZager:5/13/96
7509C:CBII-RS:JAbbotts:CM-2:Rm805A:305-6230:5/14/96
JA17\atrazine.22