



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

7625
CASWELL FILE

APR 11 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

Subject: Triazole residues in Wheat seeds and Barley grains.

From: Alberto Protzel, Ph.D.
Review Section III
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Alberto Protzel 4/10/91

To: Richard D. Schmitt, Ph.D., Chief
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Thru: James N. Rowe, Ph.D., Head
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In a 1/3/90 memorandum from W.J. Hazel to R.D. Schmitt, concern for triazole-derived residues from DPX-H6573 (also known as Nustar^R) was indicated. In particular, the metabolite triazolylalanine was reported as occurring at levels of ≤ 0.05 ppm in apples, ≤ 0.09 ppm in apple juice, ≤ 0.06 ppm in grapes, and ≤ 0.9 ppm in grape juice. In addition, triazole itself (IN-H9933; 1H-1,2,4-triazole) was found to occur at ≤ 0.14 ppm in milk, ≤ 0.03 ppm in ruminant muscle, and tentatively, at ≤ 0.004 ppm in poultry tissues and eggs. It was thus requested that the HED Metabolism Committee address the issue of whether triazole-derived residues are of toxicological concern (Issue 5).

Issue 5 was addressed in a 8/21/90 memorandum from W.J. Hazel to S. Lewis and R. Engler. Concerning Issue 5, consensus was reached that triazole-containing compounds derived from DPX-H6573 (Nustar^R) are not of concern due to their natural occurrence and/or their low toxicity and/or their indistinguishability from background.

Likewise, in a 5/8/87 memorandum to L. Rossi, A. Katz noted that "TOX Branch has determined that there is at this time no compelling toxicological basis for requiring additional metabolism studies or analytical methodologies specific for

the triazole moieties contributed by propiconazole." Levels of triazole-containing metabolites, from propiconazole were not indicated, however.

The issue of triazole-containing compounds arises again in the case of tebuconazole. As shown in Table 1, the levels of triazolyalanine that could be present jointly with tolerance levels of folicur in wheat seeds and barley grain are 6.66 and 40.0 ppm, respectively. These values are 7.4 and 44.4 times higher than the possible 0.9 ppm level for triazolyalanine in grape juice, and even higher for the other commodities listed above.

Table 1. Levels of tebuconazole and selected metabolites in various RACs.

Compound	Percent of total radioactivity	ppm possible at tolerance for RAC ¹	Reference
-----Wheat Seeds-----			
Folicur	6.0	0.5 (By defn.)	Otakie (Undated)
Triazolyalanine	80.0	6.66	
Triazolyacetic ac.	13.0	1.08	
-----Barley Grain-----			
Folicur	-	3.0 (By defn.)	Otakie (Undated)
Triazolyalanine	-	40.0	
Triazolyacetic ac.	-	6.5	

1. Triazolyalanine may occur at the following lower levels in other RACs: apples (≤ 0.05 ppm), apple juice (≤ 0.9 ppm), grapes (≤ 0.06 ppm), and in grape juice (≤ 0.09 ppm). It was not indicated if these values are the possible values at the tolerance for folicur.

To assess the toxicological significance of the observed levels of triazolyalanine (TA) in wheat grain and barley seeds a tentative, preliminary, RfD value was calculated based on studies selected from the triazolyalanine data base of submitted studies (Caswell No. 862B). Triazolyacetic acid was not included in the calculation but would not be anticipated to contribute materially to any toxicological concern. The selected studies are summarized in Table 2.

Table 2 shows two subchronic studies, 90-day rat and 13-week dog, with respective NOEL values of 5000 and 8000 ppm. The 13-week dog study is selected for calculation of the tentative RfD because it is the only one of the two studies classified as minimum. Due to the existence of only two subchronic studies (90-day rat and 13-week dog) and the absence of chronic studies an uncertainty factor (UF) of at least 1000 must be considered. Because one of the studies (rat) is

supplementary this UF could range up to 3000. Thus, the tentative RfD for triazolyllalanine will be calculated using a NOEL of 8000 ppm from the subchronic dog study (equiv. to 200 mg/kg/day, assuming 1 ppm = 0.025 mg/kg/day for a 10 kg. dog on dry lab chow; FDA, 1975). The NOEL value of ≥ 400 mg/kg/day reported for the 28-day gavage study in rats summarized in Table 2. is consistent with the selected NOEL of 200 mg/kg/day, and suggests that the actual NOEL may be higher than 200 mg/kg/day.

Thus, for a NOEL of 200 mg/kg/day, a tentative RfD for triazolyllalanine (TA) may be estimated to be 0.2 mg/kg/day if a UF of 1000 is chosen and 0.07 mg/kg/day if a conservative UF of 3000 is chosen.

Table 2. Selected studies for tentative estimation of the RfD for triazolyllalanine (From Caswell File No. 862B; see attached one-liners).

Study	Species	Dosing/Effects	Classification
90-Day feeding. Bayer AG Inst. for Tox.	Rat	0, 1250, 5000, 20000 ppm. NOEL = 5000 ppm LEL = 20000 ppm; body wt. reduction.	Supplementary
13-Week feeding. Bayer AG Inst. for Tox.	Dog	0, 3200, 8000, 20000 ppm. NOEL = 8000 ppm LEL = 20000 ppm; body wt. reduction	Minimum
28-Day oral gavage. Bayer AG Inst. for Tox.	Rat	0, 25, 100, 400 mg/kg. NOEL ≥ 400 mg/kg	Supplementary
14-Day feeding. Bayer AG Inst. for Tox.	Rat	0, 3000, 10000 ppm in drinking water. No toxic signs. NOEL not stated.	Supplementary
Two Generation Reproduction. Cent. Tox. Lab. ICI.	Rat	0, 500, 2000, 10000 ppm. Maternal NOEL ≥ 10000 ppm. Reproductive NOEL = 2000 ppm. LEL = 10000 ppm (reduced pup weights).	Supplementary

Referring to Table 1, for humans 6.66 ppm triazolyllalanine in the diet would correspond to 0.08 mg TA/kg/day in a diet (assuming 1 ppm = 0.025 mg/kg/day for a 60 kg man; FDA, 1975) consisting of 50% wheat seeds. This value is below or at the estimated RfD for TA.

Likewise, referring to Table 1, for humans 40 ppm triazolyllalanine in the diet

would correspond to 1 mg TA/kg/day (assuming 1 ppm = 0.025 mg/kg/day for a 60 kg man; FDA, 1975) in a diet consisting of 100% barley seeds. This value would drop to 0.1 mg TA/kg/day in the more likely event that the diet consists of 10% barley seeds. This value is at or below the estimated RfDs for TA.

We conclude that the levels of triazolyl metabolites in the cases of wheat seeds and barley grain do not appear to present any toxicological concern at this time. It is noted that triazole is a tebuconazole metabolite in rats (EPA MRID Nos. 409959-11 and 409959-12); while triazolylalanine, triazolylacetic acid or triazolylactic acid are not listed among the identified metabolites of tebuconazole in rats, a considerable portion of the metabolites remain to be identified.

References

Otakie G.F. Undated. Memorandum. PP#9F3724/9F03818 and FAP#9H5575. Permanent Tolerance Petitions - New Chemical - Tebuconazole. Tolerance Petition Section II. Chemistry Branch I - Tolerance Support. Health Effects Division (H7509C). U.S.E.P.A.

FDA. 1975. Food and Drug Administration. Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Third Printing. Published 1959 by the Association of Food and Drug Officials of the United States.

Attachment

HED one-liners for triazolylalanine (Caswell No. 862B).

cc: Gary F. Otakie (HED)
E. Brinson Conerly (EFED)

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/SACB
TOX ONELINERS

TOXCHEM NO. 8628- Triazolyl alanine FILE LAST PRINTED: 03/25/91

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
83-3(a) Developmental Toxicity Study Species: rat ICI Central Tox. Lab. CTL/P/875; 10/13/83	Triazolyl alanine 94.8%	252132	Levels tested by gavage in Alderly Park Alpk/AP strain from day 7 to day 16 of gestation-0, 100, 300 and 1000 mg/kg. Teratogenic NOEL > 1000 mg/kg (HDT). Fetotoxic NOEL = 100 mg/kg Fetotoxic LEL = 300 mg/kg (nonossification of odontoid process Maternal NOEL > 1000 mg/kg(HDT)		Minimum 004766 005155
83-4 Reproduction-1 generation Species: rat ICI Central Tox. Lab. RR0230/F0; 9/19/83	Triazolyl alanine 48% batch 1; batch 2 unspc. purity.	252132	Pilot Study. Dose levels: 0, 150, 625, 2500, 10,000 ppm. No effects at 10,000 ppm.		Supplementary 004766
83-4 Reproduction-2 generation Species: rat ICI Central Tox. Lab. RR0255/F0; 6/21/83	Triazolyl alanine 97.8%	252132	Interim report. Dose levels: 0, 500, 2000, 10,000 ppm. No effects noted in the first 3 weeks of the study.		Reserved 004766
83-4 Reproduction-2 generation Species: rat ICI CTL/P/1168; 8/19/86	Triazole alanine, purity 97.8%; batch TLB 1207/018-024 (Y01210/003/005	265205 265206 265207 413268-03	Dietary levels: 0, 500, 2000, 10,000 ppm in Alpk:AP strain. Maternal NOEL > 10,000 ppm (HDT). Developmental NOEL = 2000 ppm; LEL = 10,000 ppm (reduced pup weights (High dose) F1B & F2A.) Additional information did not allow upgrading. Study must be repeated.		Supplementary 005841 008292
Feeding-14 day Species: rat Bayer AG Instit. Fur Tox. Germ 82662; 10/25/82	THS 2212 (Triazolyl alanine 100% purity)	252132	Range Finding. Dose levels: 0, 3000, 10,000 ppm in drinking water. No mortalities or clinical signs of toxicity in males.		Supplementary 004766
Feeding- 28 day oral Species: rat Bayer AG Instit. Fur Tox. Germ T6011644; 1/24/83	THS 2212 (Triazolyl alanine "analytically pure")	252132	Dose levels: by gavage in Wistar BOR:WISW SPF/Cpb strain, 0, 25, 100, 400 mg/kg. No mortalities or clinical signs of toxicity. Some changes in hematology, clinical chemistry, organ weights. NOEL > 400 mg/kg(HDT)		Supplementary 004766
82-1(a) Feeding-3 month Species: rat Bayer AG Instit. Fur Tox. Germ T9015049; 2/24/84	Triazolyl alanine, batch TLB-1207	252425 258416	Levels tested in BOR:WISW (SPF-CPB) strain- 0, 1250, 5000, & 20,000 ppm NOEL = 5,000 ppm. LEL = 20,000 ppm (slight reduction in male body weight gain)		Supplementary 004101 004276 Minimum 005094 005352 005841

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TOXCHEM NO. 862B- Triazolyl alanine FILE LAST PRINTED: 03/25/91

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
32-1(b) Feeding-13 week Species: dog Bayer AG Instit. Fur Tox. Germ 1-7-015-713; 4/28/86	THS 2212 97.5% a.i., Batch TLB 1207	256058	Levels tested beagle dogs - 0, 3200, 8000, and 20,000 ppm. NOEL = 8000 ppm. LEL = 20,000 ppm (reduced body wt. gain)		Supplementary 004469 Minimum 005841
34-2(a) Mutagenic-Ames Species: salmonella Bayer AG Instit. Fur Tox. Germ 1-1006005; 1/5/83	THS 2212, batch E238099	256058	Negative for mutagenic effects up to 12,500 ug/plate with and without (S-9) activation.		Acceptable 004562 Acceptable 004469
84-2(m) Mutagenic-Ames Species: salmonella Ciba-Geigy Ltd. 960187; 7/11/86	Triazole alanine; CGA 131013 Tech, 97.4% a.i.	265204	Strains tested: TA98, TA100, TA102, TA1535 and TA1537. Negative with & without activation.		Acceptable 005841
34-2(b) Mutagenic-in vitro transform. Species: BHK cells Muntingdon Res. Centre, Eng. IC1394A81153; 5/15/81	Triazolyl alanine (R152056)	072208 252132	Levels tested: 0.5, 1, 2, 4, 8 mg/ml without S9; and 1,2,4,8,16 mg/ml with S9. Positive, with and without activation		Acceptable 004562 Acceptable 004766
84-2(b) Mutagenic-DNA damage/repair Species: E. coli Bayer AG Instit. Fur Tox. Germ 82738; 1/15/83	THS 2212 (Triazolyl alanine) purity not specified	252132	Dose levels: 62.5, 125, 250, 500, 1000 ug/plate. Nonactivated-no DNA damage. S9 activated-inadequate assay.		Accep. (W S9) 004766 Unacc (no S9) 004766
34-2(b) Mutagenic-in vitro transform. Species: mice BALB/3T3 cells Ciba-Geigy Ltd. 840324; 9/12/84	Triazolyl alanine (purity not specified)	257997	Negative with metabolic activation; inconclusive without activation. Concentrations up to 1000 ug/ml. Repeat test requested.		Unacceptable 005155 005352
84-2(b) Mutagenic-DNA repair test Species: rat hepatocytes Ciba-Geigy Ltd. 160184; 7/11/86	Triazole alanine; CGA 131013 Tech 97.4% a.i.	265204	Negative.		Acceptable 005841

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TOXCHEM NO. 862B- Triazolyl alanine FILE LAST PRINTED: 03/25/91

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	CORRECTION/ DOCUMENT#
84-4 Mutagenic-micronucleus assay Species: mice ICI Central Tox. Lab. TOM4; 9/14/82	R152056 (Triazolyl alanine) purity not spec batch 02199/49	252132 072208	Dose levels: 2500, 5000 mg/kg. No toxicity, chromosomal damage, or erythropoietic effects; however, animals were dosed only once and only one sex tested.		Acceptable 004562 Unacceptable 004766
84-4 Mutagenic-micronucleus assay Species: mice Bayer AG Instit. Fur Tox. Germ T4011615; 8/9/82	THS 2212 (Triazolyl alanine) purity not spec. ("analytically pure")	252132	Weak positive response for 8000 mg/ kg at 24-hr. Study unacceptable due to lack of critical data on positive and negative controls.		Acceptable 004562 Unacceptable 004766 005352
84-4 Mutagenic-point mutation Species: salmonella Bayer AG Instit. Fur Tox. Germ 11388; 1/5/83	THS 2212 (triazolyl alanine purity unsp.)	252132	Dose levels of 20, 100, 500, 2500, 12,500 ug/plate did not induce re-typhimurium assay. Non-activated assay not evaluated due to lack of positive control.		Acceptable 004562 004469 Unacc (+ S9) 004766 Acceptable 004766
84-4 Mutagenic-point mutation Species: Ch. Hamster V9 cells Ciba-Geigy Corp. Inc. 860258; 7/11/86	Triazolyl alanine; CGA 131013 Tech 97.4% a.i.	265204 413268-01	Positive with metabolic activation; negative without activation.		Acceptable 005841 008282
84-4 Mutagenic-micronucleus assay Species: Ch. Ham. bone marrow Ciba-Geigy Ltd. 860185; 7/11/86	Triazole alanine; CGA 131013 Tech, 97.4% a.i.	265204 413268-02	Incomplete. Additional information provided. Study upgraded to Acceptable		Unacceptable 005841 Acceptable 008292
84-4 Mutagenicity-Protein syn. Species: microorganisms Ciba-Geigy Ltd. ABR-86057; 9/19/86	Triazole alanine (purity not spec.)	265208	Inconclusive. Test species: E. coli; S. cerevisiae; A. flavus. Protein synthesis inhibitory potential.		Unacceptable 005841
85-1 Pharmacokinetics Species: rat Bayer AG Instit. Fur Tox. Germ 11583; 2/24/83	C14-Triazolyl alanine, radiochemical purity 99%	252132	Dose levels: 5 mg/kg (metabolism); 10 mg/kg (whole-body autoradiography) Rapid absorption and excretion in male rats: 95 percent of administered dose was absorbed and 94.5 percent of the radioactivity measured in urine within 48 hours. None of the metabolites were identified		Acceptable 004766

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TOXCHEM NO. 862B- Triazolyl alanine FILE LAST PRINTED: 03/25/91

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
85-1 Metabolism Species: rat Ciba-Geigy Ltd. CGA 131013; 3/2/83	C14-Triazolyl alanine, radiochemical purity > 99%	252132	Dose level - approx. 50 mg/kg. Almost entirely excreted within 24 hrs; primary route-urine, secondary route-feces. Metabolites: N-acetyl, and unaltered triazolylalanine in urine.		Minimum 004766
85-1 Metabolism Species: rat Ciba-Geigy Ltd. 131013; 10/20/83	C14-D,L-Triazolyl ala- nine purity > 99%	252132	In 24 hours, 69-86% of the dose was excreted unchanged in the urine, 8-19% was excreted as the acetyl derivative in the urine. About 3% of the dose was excreted in the urine as unknown metabolites. The total fecal radioactivity accounted for 3% of the total dose. The fecal metabolites were similar to those found in the urine except for one that could not be identified.		Acceptable 004766
85-1 Metabolism Species: rat Ciba-Geigy Corp. Inc. ABR-8602-3; 3/24/86	C14-Triazolyl alanine purity > 99%	265209	Rapid absorption and elimination. 85% excretion in urine. Single oral dose: 0.56, 54.4 and 993.7 mg/kg.		Minimum 005841
85-1 Metabolism Species: rat Ciba-Geigy Ltd. ABR86041; 6/3/86 Metabolites	C14-Triazole alanine purity > 99%	265209	Major urinary metabolites identified as unchanged triazole alanine and N-acetyl triazole alanine.		Minimum 005841
81-1 Acute oral LD50 Species: dog Inst. of Tox.; Fed Rep Germany 82663; 10/14/82	Triazolyl alanine 99%	252132	Caswell # 862AA. #323 EE (CGA-64250) Only 2 dogs used on study; both vomited a portion of the test material within 4 hours of dosing.		Invalid 004766
81-1 Acute oral LD50 Species: rat Central Toxicology Lab CTL/P/600; 1/18/81	Triazolyl alnine	252132	LD50 > 2000 mg/kg (only level tested). No mortalities at 2000 mg/kg dose tested.	3	Supplementary 004766

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TOXCHEM NO. 862B- Triazolyl alanine FILE LAST PRINTED: 03/25/91

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
1-1 cute oral LD50 pecies: rat ayer AG Instit. Fur Tox. Germ 2661; 10/19/82	Triazolyl alanine	252132	LD50 > 5000 mg/kg. Fasted male rats showed increased urinary output the day after dosing.	4	Minimum 004766
1-1 cute oral LD50 pecies: mice ayer AG Instit. Fur Tox. Germ 2661; 10/19/82	THS 2212	252132	LD50 > 5000 mg/kg. No toxic signs.	4	Minimum 004766
1-1 cute intraperitoneal LD50 pecies: rat ayer AG Instit. Fur Tox. Germ 2661; 10/19/82	Triazolyl alanine	252132	LD50 > 5000 mg/kg. At 5000 mg/kg, reversible CNS effects (spastic gait, lethargy, etc.) were observed within 1 hour of dosing. The lethal dose exceeds 5000 mg/kg.		Minimum 004766

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