ANNEX B

Thiram

Addendum to the monograph

November 2002

B.6 Toxicology and metabolism

B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA 5.1)

Study received on September 2000-09-11

Detection of a supplementary metabolite, M1, dimethylamino-4-thiazoline carboxylic acid, in urine from rats treated with a single oral dose of thiram (Gay and DeMatteo, 2000, task force study)

Findings:
Radio chromatograms from urine (0-24 hr) analysed by reverse phase HPLC showed early eluting peaks A (retention time 7.14 min; relative area 2.15%) and B (retention time 10.97 min; relative area 7.48%).
Methanol extract from urine had a recovery of 78% radioactivity; peak A accounted for 2.7% of urinary radioactivity and peak B for 7.3%.
Reverse LC/MS analysis of peak A revealed a single radioactive component, co-eluting with ion M+H (m/z 164) corresponding to metabolite U1 (PM = 163) previously identified as thiazolidine-2-thione-4-carboxylic acid (McManus, 1991, task force study). Fragmentography gave a prominent daughter of m/z 164 at m/z 118 formed by the loss of a carboxyl group. Other peaks were observed resulting from the parent compound: 1) a base peak, m/z 59, SCNH ion; 2) a peak at 105 resulting from the loss of acetic acid, 3) peak m/z 87 resulting from loss of sulphur and COOH.
Normal phase LC/MS of peak B (= 7% of urinary radioactivity) revealed 2 radioactive components, each accounting for about half of injected radioactivity from which ion M+H at m/z 175 eluted with the second radioactive peak with a molecular weight of 174 corresponding to M1.
Fragmentography gave a prominent daughter of m/z 175 at m/z 129 formed by the loss of a carboxyl or thiomethine or 2 methyl and 1 hydroxyl group. Other peaks were observed: 1) an ion at m/z 114, resulting from loss of methyl group from m/z 129; 2) a peak at m/z 59 is SCNH. Thus, dimethylamino-4-thioazoline carboxylic acid M1 is present in rat urines.

Conclusion: urine of rats collected for 24 hr contained also dimethylamino-4- thiazoline carboxylic acid, M1 corresponding to 3.5% of urinary radioactivity.

Material and methods: 4 male CD Sprague Dawley rats received by gavages a single dose of thiram (500 µCi/kg bw (thiram lot n°. 2544-97-CAV, 99.8%; ^{14}C thiram lot n°. CSL-91-341-9-27, 98.3%; specific activity: 1.27 x 10^{8} dpm/g suspension and 3.91 x 10^{3} dpm/ µg thiram) at 125 mg/kg bw in corn oil. Urine were collected at 0, 24, 48, and 72 hr. Urine from the 4 rats 24 hr following treatment were pooled, mixed with methanol and freeze for 30 min, and centrigufated for 10 min. The supernatant was evaporated under nitrogen at about 35°C.
HPLC :
Precolumn: Brownlee RP-18 New Guard cartridge
Column : Maxsil 10 C18 (4.6 x250 mm, 10 µ particules)
Flow rate: 1 ml/min
Solvent system: gradient in 40 min of 0.125 M phosphate citrate buffer, pH 3.3, 100 to 70 % and acetonitrile 0 to 30%.
LC/MS :
An LC was used for final purification and introduction of samples into mass spectrometer.
Effluent from HPLC was split 4:1 to a RAM and to a MS such that radioactive metabolites were analysed by the RAM and the MS (electron spray ionisation with N_2 as drying gas) at the same time.
Reverse phase LC/MS column:
For peak A analysis: Ultracarb (2 x 150 mm, 5 µ particles) microbore.
Solvent: gradient of formic acid 100% (50 mM) to 70% within 40 min and acetonitrile, 0 to 30%.
Flow rate: 0.3 ml/min.
For peak B analysis: Zorbax Amino column (4.6 x 250 mm, 10 µ particles).
Solvent: gradient of formic acid 0 to 100% (50 mM) within 30 min and acetonitrile, 100 to 0% simultaneously and from 30 to 38 min, 100 to 0% formic acid and 0 to 100% acetonitrile.
Flow rate: 0.3 ml/min.

Isolated metabolites were chromatographed, and the RAM detected radioactive components in the effluents while full scan mass spectra were obtained in the first quadrupole, MS-1 in Quad-1 (LC/MS). From the full scan spectra the $(M+H)^+$ ion of radioactive metabolite was determined. Next, this ion was selected in the first quadrupole (MS-1 in Quad 1) and fragments were formed by collision induced dissociation in quad 2 using argon at a collision energy of 15 to 40eV; the full scan mass spectra acquired in the 3 quad yielded product ions from the dissociation of only the selected precursor ions.

Comment: in the monograph, the results from identification of urinary metabolites were reported in table B.6.3-1.
For this study, chromatographic conditions were:
Before HPLC analysis, urine samples were diluted with phosphoric acid buffer (0.01 M) pH 3, followed by filtration with Gelman acrodisc LC-13, PVDF filters. HPLC column was a Baxter Burdick & Jackson C-18 (5

µm, 4.6 x 250mm) with a precolumn (C-18, 7µ). Solvent system: 100% phosphoric acid buffer for 5 min; increase to 30% acetonitrile over next 20 min and increase to 100% acetonitrile over next 5 min.
Urinary radioactivity reached 14.5% of the dose (125 mg/kg bw) within 24 h.
Urinary metabolites were: DDC-thiosulfenic acid (5.4% of administered dose/37.3% of urinary radioactivity), DDC-alanine (4.9 %/33.9%), 2-thioxothiazolidine-4-carboxylic acid (1.8 %/12.6%), DDC-glucuronide (0.8 %/5.6%), DDC-methyl ester (0.5 %/3.4%).
A supplementary metabolite, metabolite M1, dimethylamino-4-thioazoline carboxylic acid was identified recently in urine from male rats after single high dose administration. Despite the fact that in this study, not all urinary metabolites were quantified in parallel, we estimate that 0.5% of administered dose is excreted as metabolite M1.

B.6.7 Neurotoxicity (Annex IIA 5.7)

B.6.7.1 Neurotoxicity following acute exposure in rats (Annex IIA 5.7)

A new study received in May 2002, was not requested for Annex I listing. An acute neurotoxicity study was summarized in the monograph (Driscoll and Hurley, 1993). The NOAEL (5 mg/kg bw/d) of this study was used for setting the ArfD. In this study, acute neurotoxicity was evaluated at 5, 150 and 600 mg/kg bw.
The purpose of this new study was to further investigate the neurotoxic potential of thiram at doses between the existing NOAEL (5 mg/kg bw) and LOAEL (150 mg/kg bw) determined in the previous reported study.

In the previous study, reported in the monograph, behavioural function was evaluated using a screening battery of tests (FOBs). A NOAEL of 5 mg/kg bw was based on FOB findings. There were no treatment-related findings in either sex of the high dose group for gross or microscopic evaluations of the nervous system.
Motor activity was also reported in the study of Driscoll and Hurley (1993) but not discussed in the monograph: both males and females in the 150 and 600 mg/kg bw dose groups were found generally less active at each of the three post-treatment assessment periods (3.5 hour, 7 and 14 day post treatment). Body weight was altered at 150 and 600 mg/kg.

Table B.6.7-1 Summary of neurotoxic effects reported in the first study (Driscoll and Hurley, 1993)

Doses/	**0**		**5**		**150**		**600 mg/kg bw**	
effects	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Body weight								
Day 1					↓6%		↓6%	
Day 7					↓4%		↓12%	↓6%
Day 14							↓5%	
Motor activity								
3.5 hour	509±205	854±338	570±208	750±284	403±103	359±98**	335±138*	337±70**
7 day	728±284	1441±591	676±237	1333±872	631±240	731294**	548±191	540±202**
14 day	857±342	1416±501	798±249	1265±422	575±193**	887±490**	629±286*	748±403**
Rel Brain weight	0.625±0.033	0.946±0.063	0.621±0.023	0.909±0.0523	0.598±0.020	0.934±0.067	0.622±0.036	0.907±0.039
FOBs					Effects (see monograph)			

** Significantly different from control p<0.01
* Significantly different from control p<0.05

Acute neurotoxicity study in rats (Williams, 2001)

<u>Findings:</u>

Body weight was significantly lower for both sexes in the 150-mg/kg bw groups on day 8 and 15. Food consumption was reduced for males in the 60 and 150 mg/kg bw group and for females in the 25, 60 and 150 mg/kg bw group during week 1.
Overall motor activity was reduced in the top dose group females on day 1, 8 and 15. Males were unaffected. Altered relative brain weight as observed in male rats at 60 mg/kg bw is considered as a neuro-anatomical endpoint. However, this effect was not observed at higher doses and is therefore considered as incidental (Table B.6.7-2).

Table B.6.7-2 Neurotoxic effects reported in the second study (Williams, 2001)

Doses/ Effects	**0**		**10**		**25**		**60**		**150 mg/kg bw**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Clinical signs										
			No compound related effects							
Body weight										
Day 1										↓4%
Day 8							↓5%	↓3%	↓8%	↓5%
Day 15								↓6%	↓6%	↓5%
Food consumption										
Week 1						↓16%	↓20%	↓19%	↓27%	↓22%
Motor activity										
Pre study	169 ±113	261± 86	113±72	321±126	211±74	305±53	201±67	325±157	230± 124	370± 97
Day 1	194 ±63	213± 66	198±112	224±126	149±25	213±122	147±76	146±65	143± 48	120± 112*
Day 8	155 ±74	404± 181	167±54	466±150	160±67	365±108	137±63	308±118	131± 58	277± 120*
Day 15	224 ±99	416± 146	294±114	441±174	282±90	288±169	154±114	355±137	186± 56	244± 140*
Rel Brain weight	1.76	1.72	1.78	1.74	1.78	1.74	1.83*	1.72	1.80	1.74

Conclusion: NOAEL neurotoxicity = 60 mg/kg bw based on reduction of overall motor activity at 150 mg/kg bw.

Guidelines:
Experimental protocol partly in compliance wit OECD guidelines 424 (1997)
This study was performed as a complementary experimental study: only total motor activity was measured without documenting a specific clinical motor activity as measured/observed in the previous study. As there were no treatment-related findings in either sex of the high dose group for gross or microscopic evaluations of the nervous system in the first study, no gross or microscopic examination was preformed in this study.
GLP status: Yes
Material and methods:
53 male and female rats (Alpk: APSD) received a single oral dose of thiram technical (Batch n° V777R/G9605676; 98.7%) in corn oil at 10, 25, 60 or 150 mg/kg bw as a single dose by gavage, on day 1.
The study is acceptable.

B.6.10.3 Establishment of an Acute Reference dose (ARfD) (Annex IIA B.5.10)

During ECCO 78, it was proposed to derive an ARfD for thiram from the acute neurotoxicity study. However, a large spacing between the NOAEL and LOAEL in this study led the applicant to perform a new study in which this gap was filled in. Based on the results reported in the second acute neurotoxicity study, a NOAEL for acute neurotoxicity = 60 mg/kg bw is acceptable. Applying an assessment factor of 100 gives a

ArfD= 0.6 mg/kg bw/d.

VITAVAX 200 FF (Data received in May 25, 2000)

B.6.15a Exposure data

B.6.15.1a Estimation of operator exposure (Annex IIIA 7.2.1.1)

Exposure to seed treatment chemicals during seed treatment and sowing is largely a function of the operations undertaken. As such a generic approach can be taken.
In the monograph, the RMS used for estimation of operator exposure direct extrapolation from the four SEEDTROPEX studies. However, total potential dermal and potential inhalation data were used. The RMS concluded that operator exposure was not acceptable.
Uniroyal Chemical was asked to clarify some points with respect to the SEEDTROPEX calculations.

12/10/99 – Clarification from Uniroyal Chemical:

The SEEDTROPEX computer model for seed treatment calculates operator exposure at two levels:
(a) Total potential dermal exposure, the exposure that would be expected on the outer garments. This was the measured contamination on a loose, two-piece cotton garment consisting of a loose jacket with loose sleeves and loose collar, over loose cotton trousers with loose cuffs at the ankle.
The RMS used this level.
(b) Estimated actual dermal exposure: the exposure that would be expected on the skin if only one layer of clothing as detailed in (a) above was worn. This was measured by sampling cotton underclothing in the study.

The notifier proposes to use estimated actual dermal exposure data (b) as being more representative of exposure in reality.
This approach permits to make also an estimation of exposure with protective clothing.

It is proposed by the notifier to use the following protective clothing:
- Protective coveralls (TO EN 466) reduction coefficient: 0.05
- Protective gloves: reduction coefficient: 0.01
- Disposable filtering respirator (TO EN 149 FFP 3) reduction coefficient: 0.08

The above clothing is worn over the normal working clothing. Therefore, the clothing worn in practice will offer much higher levels of protection than are estimated in the SEEDTROPEX model.

A new estimation of operator exposure is proposed without PPE (Table **B.6.**15.1a-1), using in a second approach a coverall (Table **B.6.**15.1a-2) and in a third approach a coverall and a disposable filtering respirator (Table **B.6.**15.1a-3).

Table B.6.15.1a-1 Estimation of operator exposure.

SEED TREATMENT WORKED EXAMPLE (calculated using generic exposure values)							
TASK	**Total potential dermal exposure (mg/op)**	**Estimated actual dermal exposure (mg/op)**	**Inhalation exposure (mg/op)**	**Frequency operation/ Day**	**Total potential dermal exposure (mg/day)[1]**	**Estimated actual dermal exposure (mg/day)[2]**	**Inhalation exposure (mg/day)[1,2]**
Calibration	6.51	2.85	0.040	1	6.51	2.85	0.040
Mixing/ Loading	1.04	1.04	0.026	1	1.04	1.04	0.026
Bagging off 25 kg (mg/hr)	2.67	0.862	0.131	8	21.3	6.90	1.045
Cleaning	174	16.67	1.152	1	174	16.67	1.152
Total exposure (mg/person/day)					203	27.5	2.26
Total exposure (mg/kg *bw/d)					2.9	**0.392**	**0.032**

[1] : values used in the monograph for estimation of dermal exposure.
[2]: values proposed to be used by the notifier for estimation of dermal exposure.
* Body weight = 70kg

From the table B.6.15.1a-1, using a dermal absorption of 10%, a dermal absorbed dose = 0.0392-mg/kg bw/d is calculated.
Total absorbed dose (dermal absorbed dose + inhalation exposure) = 0.0712 mg/kg bw/d = 356% of AOEL. In these conditions, exposure is not acceptable.

In a second approach, operator exposure is estimated when protective coverall is worn (Table B.6.15.1a-2).

Table B.6.15.1a-2 Estimation of operator exposure: Protective coveralls can be worn F = 0.05

TASK	**Total potential dermal exposure (mg/op)**	**Estimated actual dermal exposure (mg/op)**	**Inhalation exposure (mg/op)**	**Frequency operation/ day**	**Estimated actual dermal exp. +coverall**	**Inhalation exposure (mg/day)**
Calibration	6.51	2.85	0.040	1	0.142	0.040
Mixing/ loading	1.04	1.04	0.026	1	0.052	0.026
Bagging off 25 kg (mg/hr)	2.67	0.862	0.131	8	0.345	1.045
Cleaning	174	16.67	1.152	1	0.833	1.152
Total exposure (mg/person/day)					1.372	2.26
Total exposure					**0.0196**	**0.032**

(mg/kg *bw/d)						

* Body weight = 70kg

From Table B.6.15.1a-2, a dermal absorbed dose = 0.00196 mg/kg bw/d is calculated giving a total absorbed dose = 0.0339 mg/kg bw/d = 169 % of AOEL. This estimation gives results above AOEL and is not acceptable.

In a third approach, estimation of operator exposure is assessed using protective coverall and disposable filtering respirator (Table B.6.15.1a-3).

B.6.15.1a-3 Estimation of operator exposure: Protective coveralls (F= 0.05) + protective gloves (F= 0.01) and disposable filtering respirator (F = 0.08)

TASK	**Total potential dermal exposure (mg/op)**	**Estimated actual dermal exposure (mg/op)**	**Inhalation exposure (mg/op)**	**Frequency operation/ Day**	**Estimated actual dermal exp. +Coverall + gloves**	**Inhalation exposure (mg/day) + Respirator**
Calibra Tion	6.51	2.85	0.040	1	0.00142	0.0032
Mixing/ Loading	1.04	1.04	0.026	1	0.00052	0.0020
Bagging off 25 kg (mg/hr)	2.67	0.862	0.131	8	0.00345	0.0836
Cleaning	174	16.67	1.152	1	0.00833	0.092
Total exposure (mg/person/day)					0.01372	0.933
Total exposure (mg/kg *bw/d)					**0.000196**	**0.0133**

* Body weight = 70kg

From table B.6.15.1a-3, a dermal absorbed dose = 0.000019 mg/kg bw/d is calculated.

From these results, a total absorbed dose of 0.0133-mg/kg bw/d is obtained which represents 67 % of AOEL. Exposure of operator is acceptable if protective equipment is worn such as coverall, gloves and disposable filtering respirator.

Conclusion:
The exposure calculations presented in table B.6.15.1a-1, B.6.15.1a-2 and B.6.15.1a-3 can still be considered as worst case for the following reasons:

1) A high degree of automation is present in many seed treatment factories, especially in the large high throughput situations.
2) Many seed treatment products are sold in drums with snap-lock dry break couplings, which reduce exposure during mix/load operations.
3) Bagging operations are frequently automated. The above calculations assume eight hours constant exposure per day. Actual exposures would be much lower.
4) Cleaning operations are not always performed each day. Operatives will change into specific clothing for this operation. Uniroyal recommends the following minimum protective clothing: coverall with integral hood, cap, gauntlet style gloves or disposable forearm covers, boots, filtering respirator.
5) SEEDTROPEX assumes that one operative conducts all the tasks: calibration, mix/load, bagging and cleaning. This is not what actually happens, as one man cannot perform all the tasks. In most seed treatment

plants the tasks are divided between several operatives and in the larger high throughput plants some tasks such as bagging are automated. It is therefore more correct to divide the tasks.

The above calculations demonstrate that in the worst case, assuming that one operative conducts all the tasks, seed treatment uses are safe for the operator when the appropriate protective clothing is worn.

THIRAM 80 WG Data received: October 13, 1999.

B.6.15.3b Estimation of bystander exposure (Annex IIIA 7.2.2)

Dermal exposure can be assumed to be a result of drift, incidental and for a very short time (1-min).
Exposure during spraying of high crops represents the worst-case situation. Usually, clothes are worn with a total of uncovered areas of 0.4225 m^2. Assumption is made that 100% of applied dose is deposited: 3.2 kg a.i./ha = 320 mg/m^2.
Spray drift deposition is based on Ganzelmeier et al (1995), estimated to be 2.6% at 7.5 m distance in avineyard.
Dermal exposure of the bystander is as follows:
D = 100% deposition x drift deposition x exposed area
= 320 mg/m^2 x 0.026 x 0.4225 m^2/person/day
D = 3.5 mg/person/day.
This is much lower than the Dtol = 133 mg/person/day as calculated in the German Model.
Inhalation exposure:
The inhalation exposure based on that of the operator exposure is:
I = I_A x work rate x application rate
I_A = specific exposure application (tractor mounted) = 0.018
I = 0.018 mg/person x 8 ha/day x 3.2 mg/kg a.i. /ha
I = 0.46 mg a.i./person/day.
The exposure time for the bystander will be very short and it is assumed that the passing time of the spraying tractor will be 1 min. I must be corrected for actual exposure.
I = 0.46 mg a.i./person/day/360 = 0.00127 mg a.i./person.
I is much lower than the I_{tol} = 1.33 from the appropriate calculation of the German Model.

The above dermal and inhalation exposures for bystanders indicate an acceptable risk.

B.6.15.4b Estimation of worker exposure (Annex IIIA 7.2.3.1)

Re-entry exposure is predominantly via dermal route (contact with foliage).
Residues on the foliage depend on:
- Application rate
- Extent of remaining residues from previous applications
- the crop habitat

Transfer of residues from foliage to clothes or skin of workers is more or less independent of the product applied, but depend mainly on the intensity of contact with foliage.
The foliar dislogeable residue can be calculated using a default value of 1 µga.i. /cm^2 for an application rate of 1 kg a.i./ha according to: 1 kg a.i./ha = 10 µg/cm^2; two sided leaves.

In high crop, two application schemes are used:
- Orchard: 4 applications/season at 2.4 kg a.i./ha
- Vines: 3 applications /season at 3.2 kg a.i./ha.

Dermal exposure (µg a.i./person/day) = DFR (dislogeable foliar residue/kg a.i./ha) x TF (transfer factor (cm^2/person/h) x WR (work rate h/day) X P (penetration factor clothing: w/o PPE = 1)

Orchard:
D =4 x 1 µg/cm^2 x 30000 cm^2/person/h x 8 h x 2.4 kg a.i./ha x 1 = 2304 mga.i. /person/day
This value exceeds the Dtol = 133 and is therefore not acceptable.
When using PPE:
D = 4 x 1 µg/cm^2 x 30000 cm^2/person/h x 8 h x 2.4 kg a.i./ha x 0.01 = 23. 04 mg a.i./person/day
This value is lower than D_{tol} and therefore, dermal exposure is acceptable for workers when PPE are worn.

Vine:

D =3 x 1 µg/cm^2 x 30000 cm^2/person/h x 8 h x 3.2 kg a.i./ha x 1 = 2304 mg a.i. /person/day
This value exceeds the Dtol = 133 and is therefore not acceptable.
When using PPE:
D =3 x 1 µg/cm^2 x 30000 cm^2/person/h x 8 h x 3.2 kg a.i./ha x 0.01 = 23. 04 mg a.i./person/day
It can be concluded than worker exposure is acceptable when PPE are worn.