UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE: March 10, 1978

SUBJECT: Products containing cis or trans 1- (3-chloroallyl)-3,5,7 triaza-1-azoniaadamantane chloride

FROM: K. L. Bailey, Ph.D
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K L Bailey

Cas. No. 151

TO:
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THRU: Chief, Toxicology Branch

(Note: For further pertinent details consider the K. Bailey memo dated Oct. 27, 1977, concerning this chemical.)

I. Conclusion:

A. We are concerned with two separate and distinct compounds, the cis and trans isomers of the active ingredient.

While it is reasonable to accept data generated using an essentially 50/50 mixture of the two compounds, it is by no means clear that data generated using the cis compound alone are adequate to support the registration of both the cis and trans compounds.

At such time as it is pertinent, this point must be clarified.

B. A teratogenic study will be required to support the registration of the cis and/or trans compound, at such time as it is pertinent.

C. A reproduction study with some emphasis directed toward the male will be required using the cis and/or trans compound, at such time as it is pertinent.

This consideration is based upon decreased spermatogenesis and decreased testicular weight observed in a rabbit 21-day dermal study. (Accession No. 003579)

D. Until such time as a safe human exposure level can be determined, human exposure should be kept as low as feasable.

This consideration is based upon the following:

1. Testicular effects in a rabbit

21-day dermal study (NOEL = 33 mg/kg/day)
Accession No 003579

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2. Liver damage in a dog

90-day oral study, NOEL = 7.5 mg/kg/day (Accession No. 230645)

E. It is not clear what if any data is associated with the Rabbit 3-Week Dermal Application Study - Microscopic Observations (Accession No. 140001). This point must be clarified at such time as it is pertinent.

F. The LD_{50} (>2 gm/kg) reported in the Rabbit dermal LD_{50} study (Accession No. 003631) is at variance with the LD_{50} (400 mg/kg) reported under Accession No. 003579. At such time as it is pertinent, this point must be clarified.

G. While these are many toxicologic problems associated with the cis and/or trans compound (See K.L. Bailey, Oct. 18, 1977 Memo concerning a milk preservation tablet), there is no concrete toxicologic data at hand that suggests that this compound is a candidate for a RPAR referral, at present.

II. Toxicology Review

A. Accession No. 003579 (EPA Reg. No. 464-375)

The material used to perform the various toxicological studies considered here in Cinaryl 200 which is cis i-(3-chloroallyl) - 3,5, 7 -triaza -1-azoniaadamantane chloride.

1. Data Summarized But Not Reported in Full

a. Rabbit Eye Irritation Study

In a study using 3 rabbits, incorporating both washed and unwashed eyes, the compound was found to be minimally irritating.

b. Rabbit Dermal Irritation Study

In a study involving prolonged dermal exposure (14 days, unabraded skin and 3 days, abraded skin) the compound was found to be irritating especially when applied to abraded skin.

c. Human Skin Sensitization Study (Conducted by Hill Top Research Inc.)

In this study, for which no actual results were supplied, 72 human test subjects were repeatedly (9x) exposed for 24 hours to .5 ml of a 1 solution of the compound. Considering the lack of dermal irritation observed following administration of the various solutions it was concluded the test solution is minimally irritating and does not induce sensitization.

d. Acute Oral LD_{50} Studies

Animal (Sex)	Preparation Fed	LD_{50} (g/kg)
Rat (Female)	10% aqueous solution	1.07
Rat (Male)	10% aqueous solution	0.94
Rabbit (Mixed)	6.3% aqueous solution	0.04
Chicks (Male)	Undiluted (Capsules)	2.80
Cavies (Males)	10% aqueous solution	0.71

2. Studies Reported in Full

a. Rabbit Acute Dermal LD_{50} Study (Conducted by Industrial Bio-Test)

In this study 4 groups of rabbits, each group composed of 4 rabbits, were respectively exposed dermally to 267, 400, 600 and 900 mg/kg of the test compound using a 50% (w/v) aqueous solution. Hypoactivity and emaciation were noted. Necropsy did not reveal any significant gross pathological changes in those animals which died during a 14-day post-exposure period. The observed dermal LD_{50}, 400 mg/kg, places the compound in Tox. Cat. II in this Core Minimum Study.

b. Rabbit 21-Day Subacute Dermal Toxicity Study Conducted by Industrial Bio-Test

In thus study, using adult New Zealand albino rabbits, the following dosage regimen was used:

Study	Group	Number of Animals Male	Female	Condition of Skin	Dose (mg/kg/day)*	No. of Applications
I	Treated Control -1	5	5	Intact	-	15**
	Test-1	5	5	Intact	10.0	15
	Test-II	5	5	Abraded	10.0	15
	Test-III	5	5	Intact	25.0	15
	Test-IV	5	5	Abraded	25.0	15

Study	Group	Number of Animals Male	Female	Condition of Skin	Dose (mg/kg/day)*	No. of Applications
II	Treated Control-II	5	5	Intact	-	15**
	Test-V	5	5	Intact	50.0	15
	Test-VI	5	5	Abraded	50.0	15
	Test-VII	5	5	Intact	100.0	15
	Test-VIII	5	5	Abraded	100.0	15

*Doses are expressed in terms of Cinaryl 200 and not in terms of the 20.0 per cent (w/v) aqueous solution as prepared.

**Animals in this group received daily dermal applications of tap water comparable in volume to the amount of test solution administered to the high dose groups.

The following parameters were measured:

1. Mortality
2. Body Weight Changes
3. Hematologic and Clinical Blood Chemistry Values
4. Results of Urine Analysis
5. Gross and Microscopic Oathological Findings

With the exception of the lowest dosage group, the following tissues from rabbits in all were examined histologically:

Heart, aorta, trachea, lungs, liver, gall bladder, pancreas, esophagus, stomach, small intestine (duodenum, jejunum, ileum and caecum), colon, spleen, lymph nodes (mediastinal and mesenteric), kidneys, urinary bladder, gonads, prostate, seminal vesicles, uterus, pituitary, adrenal glands, salivary glands, thyroid gland, parathyroid glands, skeletal muscle (thigh), sternum, peripheral nerves (sciatic and femoral), brain, and skin of application site. All sections were stained with Hematoxylin and Eosin.

While urine was supposedly analized, no results were mentioned or presented. This is judged to be a minor defect.

Of the parameters measured, the only unambiguous changes noted are as follows:

(a) A Significant decrease in both relative and absolute testes weight at 100 mg/kg with a NEL of 50 mg/kg.

(b) Decreased spermatogenesis at 100 and 50 mg/kg with a NEL of 25 mg/kg.

4

As the compound affects spermatogenesis, it is reasonable to inquire as to whether it has an effect upon reproduction. At such time as it is pertinent, a suitable reproduction study should be initiated to determine what if any effect the compound has upon reproduction.

B. Accession No. 050088 (EPA Reg. No. 464-403)

The material used to perform the studies considered herein is Dowicil 100 which is [redacted] mixture of the cis and trans isomers of 1-(3-chloroallyl)-3,5,7 triaza -1- azoniaadamantane chloride.

1. Rat 90-Day Feeding Study (Conducted by Dow)

At present, it is not possible to assign any significance to this study for the following reasons:

(a) The compound has a marked effect upon food consumption thus yielding significant compound related decreases in body weight at all dosage levels (7 through 60 mg/kg). Thus, it is impossible to evaluate the significant increase in testicular to body weight ratio noted at all dosage levels.

(b) The deleterious effects noted under gross and microscopic observations for female rats are in all cases reported to be of greater incidence in the control than in the experimental group suggesting that the control group is not suitable.

(c) There appears to be a significant incidence of intestinal parasitic infestation. It is not clear if this did or did not modify the results.

C. Accession No. 003581 (EPA Reg. No. 464-375)

This study was performed using Dowicil 200 (Formally Cinaryl 200) which is 97% 1-(3-chloroallyl) -3,5,6 - triaza -1- azoniaadamantine chloride.

1. Rabbit 91-Day Dermal Study

This study is, in essence, a continuation of the 21-Day Rabbit Dermal Study found under Accession No. 003579. No compound related effects are noted in this study.

Based on the 21-day study, this is what one would expect. Thus, no new [illegible] dose used in this study) for spermatogenic effects. The same basic considerations identified in the reivew of the 21-day study apply to this study as well.

D. Accession No. 230645 (EPA Reg. No. 464-403)

The material used to perform this study was Dowicil 100 which is [redacted] mixture of the cis and trans isomers of 1-(3-chloroallyl)-3,5,7 triaza -1- azoniaadamantane chloride.

5

1. Dog 90-Day Oral Study - Conducted by Dow Chemical

In this study, 4 groups of dogs, each group composed of 4 males and 4 females, orally received via capsule dose levels of 0 (control), 7.5, 15 and 30 mg/kg/day of the test compound.

The following parameters were evaluated:

a. Appearance and Behavior
b. Body Weight and Food Consumption
c. Hematology
d. Blood Chemistry
e. Urinalysis Values
f. Ophthalmologic Findings
g. Tissue Weight
h. Gross and Microscopic Pathology of a wide and adequate variety of tissues in the control and high dosage groups. In the intermediate dose groups only liver, urinary bladder, kidneys and a rib with bone marrow were examined microscopically.
i. Mortality

A very brief synopsis of the kinds of compound related effects noted ~~is~~ are as follows:

	mg DOWICIL 100 kg/day					
	Males			Females		
Alterations in:	7.5	15	30	7.5	15	30
Body Weight[a]	no	no	no	no	no	no
Food Consumption[b]	no	no	no	no	no	no
Survival	no	no	no	no	no	yes(½)
Overt signs of toxicity	no	no	no	no	no	yes
Hematology[c]	no	no	yes	no	slight	yes
Clinical chemistries[d]	no	no	no	no	no	no
Urinalysis[e]	no	no	no	no	no	no
Organ weights[f]	no	yes	yes	no	slight	yes
Gross pathology[g]	no	no	no	no	no	yes
Histopathology[h]	no	no	no	no	yes	yes

Of the compound - related effects noted, the following appear to be the most significant:

a. Obliterative vasculitis and perivasculitis of selected hepatic blood vessels in females in the 15 mg/kg/day group.

b. Moderate perivascular and pericholangiolar infiltration of mononuclear cells in the liver of 4/4 males and 3/4 females in the 30 mg/kg/day group.

c. Hyperplasia of the reticuloendothelial cells lining the hepatic sinusoids of the liver in 4/4 males and 3/4 females in the 30 mg/kg/day group. The observable NEL based on liver damage is 7.5 mg/kg in this core minimum study.

E. Accession No. 003631 EPA File Symbol 464-UNG

The material used to perform the following studies was Dow XD-1840 Preservative which is composed of [redacted] cis-trans) and sodium bicarbonate.

(Note: All of the following studies were conducted by Dow)

1. Acute Rat Oral LD_{50} Study

In this study 3 groups of rats, each group composed of 4 female rats, were respectively exposed to .5, 1 and 2 gm/kg via oral intubation; the observed oral LD_{50} is 1.2 gm/kg (Tox. Cat. III, Core Minimum).

2. Rabbit Eye Irritation Study

In this unwashed - eye study, right eye of 6 rabbits was exposed to .1 gm of the compound. (Tox. Cat. III, Core Minimum)

3. Rabbit Dermal Irritation Study

In this study, 2 separate groups of rabbits were dermally exposed respectively to a 10% aqueous solution of the compound and to the solid powder. Irritation is minimum in the case of unabraded skin and marked in the case of abraded skin. (Tox. Cat. II abraded, Tox. Cat. IV unabraded, Core Minimum)

4. Rabbit Acute Dermal LD_{50} Study

The value obtained in this study, $LD_{50} > 2$ gm/kg, are at variance with that reported in the data listed under Accession No. 003579, (LD_{50} = 400 mg/kg). Clarification is required.

5. Rabbit 25-Day Dermal Toxicity Study.

This study is designed to determine what concentration of the active ingredient may safely be used. The study is severely compromised by the small number of animals used, 2 of each sex per group, and by the fact that no consideration is given to the results obtained in previous studies. Specifically, testicular arrest is noted in 1 of 2 high-level male and focal hepatic necrosis in 1 of 2 high-level females. Equivalent results have been found respectively in a subacute rabbit dermal study

[redacted]

F. Accession No. 140001 EPA Reg. No. 464-375, 464-327.

The material used to perform the studies associated with this accession number 140001, is Dowicide Q which is [redacted] of the cis and trans isomers of 1 - (3-chloroallyl) -3,5, 7 triaza -1- azoniaadamontane chloride.

1. Human Skin Irritation and Skin Sensitization Study - Performed by Industrial Bio-Test.

   In this study, 50 human subjects were repeatedly exposed simultaneously to both a .5% aqueous solution of the ingredient and to a dilute cutting oil emulsion (.5% active) of unknown composition. While the actual volume is not specified, the volume applied may be roughly estimated as .3 ml.

   The following application schedule was used:

   a. Apply patch, wait 24 hours and score.

   b. Allow tissue to rest 24 hours and repeat step above.

   c. Repeat the above, a and b, and wait 14 days following the 9th application.

   d. Apply a final "test" patch, wait 24 hours, and score the result.

   Following this procedure, no signs of either dermal irritation or sensitization were evident.

   Based on this study and other studies, the compound is judged not to be either dermally irritating or a sensitizer at a concentration of .5% or below.

2. Rabbit 3-Week Dermal Application Study - Microscopic Observation.

   It is not clear if the microscopic examinations reported here were associated with a study already considered or are associated with some study not submitted, as yet. This data cannot be adequately reviewed until the associated experimental results are presented. However, the results indicate heart, liver and kidney effects when 15 cc of a 5% aqueous solution is applied dermally to rabbits 5 days per week for 3 weeks and heart and kidney effects when a 1% solution is used. It is not possible to establish a NEL as effects are noted at the lowest concentration used, 1%.

III LABORATORIES PERFORMING TOXICOLOGIC TESTS

Lab	Test	Reference
IBT	Rabbit Acute Dermal	Accession No. 003579
IBT	Rabbit 21-Day Dermal	
Hill Top Research	Human Sensitization	
Dow	All Other Studies	
Dow	All Studies	Accession No. 003631
Dow	All Studies	Accession No. 050088

IV LABORATORIES PERFORMING TOXICOLOGIC TESTS

Lab	Test	Reference
Dow	All Studies	Accession No. 003581
IBT	Human Skin Irritation and Sensitization	Accession No. 140001
	Summary of Other Studies -Source not clear	
Dow	All Studies	Accession No. 230645

J.E.W 5/9/78

Typist: TH