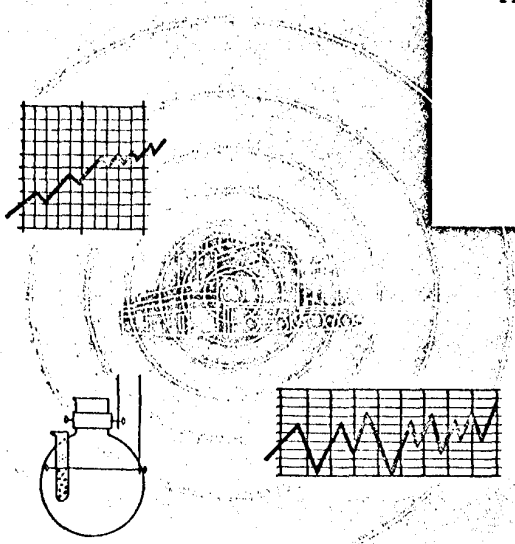
Monsanto

THE TOXICITY OF PHOSPHATE ESTER PLASTICIZERS, A QUALITATIVE "RISK-BENEFIT" ANALYSIS

PCD #0004
O.D. 1569

P. R. Graham
August 20, 1973



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COMPANY

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III. CONCLUSIONS

1. Historically, phosphate ester plasticizers, as a class, have been considered relatively non-toxic. Santicizer 141 is FDA regulated for food packaging by virtue of two-year chronic oral feeding studies. Acute oral toxicity studies (LD_{50}) show phosphate ester plasticizers to be relatively non-toxic.

2. However, the literature is replete with studies, on both humans and animals, showing disastrous effects of tri-o-cresyl phosphate. Most of this information reveals neurological problems. Both TCP and Santicizer 140 cause demyelination (degeneration of nerve sheath) and ~~inhibit cholinesterase activity.~~ Although S-140 has been shown to absorb through the skin of rats and cause cholinesterase inhibition, the literature points to the critical mono- and di-ortho cresylic acid structures as being prime causes; our results appear to be the same regardless of the cresylic acid source. The "low ortho" Pitt-Consul and the "high ortho" Merichem cresylic acids both give similar results. Santicizer 141 (2-ethylhexyl diphenyl phosphate), Santicizer 148 (isodecyl diphenyl phosphate) and triphenyl phosphate do not cause demyelination according to our tests. Kronitex 100 (isopropyl phenyl diphenyl phosphate) does cause demyelination.

3. All of the phosphate esters are toxic to fish. Santidizer 148 appears to be the least toxic, followed by Santicizer 141. TCP, S-140, K-100 and TPP are moderately to highly toxic to fish according to this test.

4. However, the fish toxicity hazard could conceivably be modified by the biodegradation rates of these esters. TCP and TPP are the most biodegradable. S-140 and S-141 follow. K-100 and S-148 are the least biodegradable.

5. Santicizer 141 has exhibited dermatological problems to humans.

6. No vapor inhalation hazards are anticipated with any of the phosphate esters.

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7. Generally, it is concluded that all of the phosphate esters are considerably more dangerous than the phthalate ester class. The fact that these esters are not used in the high quantities as the phthalates has minimized interest in their toxicity. As it becomes known that these esters are being used in greater amounts for applications where skin absorption is a definite possibility (upholstery), we can expect a rash of studies pointing to these hazards.

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V. DETAILS

A. Toxicological History of Phosphate Esters

The literature is replete with studies pointing to the generally toxic nature of phosphate esters relative to both humans and animals. By far, the most dangerous member of this class of compounds, these reports show, is tri-o-cresyl phosphate. Being a poison for the nervous system, serious and even fatal accidents have occurred. An outbreak of the so called "ginger paralysis" caused by the adulteration of Jamaica ginger, occurred in the U.S. in 1930. Some 10,000 North Africans were paralyzed in 1959 through the ingestion of cooking oil contaminated with ToCP. A number of problems involving smaller groups of industrial workers was also reported.

As a consequence of these epidemics, extensive studies of the specific effects of phosphate esters on animals have been made. Oversimplifying, o-cresylic phosphate esters affect the nervous system in two ways. First, they attack the cells of the myelin which is a fatty sheath covering nerve fibers causing them to become exposed and damaged. Secondly, these esters inhibit cholinesterase activity. Acetyl choline is needed to conduct electrical impulses throughout the nervous system. Cholinesterase enzyme is required to deactivate acetyl choline immediately after impulses have been transmitted to prohibit the repetition of the message resulting in convulsions and other involuntary effects.

It is still not clear as to the exact structures which cause this toxicity. Mono- and di-orthocresol derivatives have paralyzing activity. Actually, the mono-orthocresyl ester was six times more toxic to animals than the tri-o-cresyl compound. The pure ToCP compound attacks only the peripheral nerves while the mono- and diortho cresylic-esters attack the pyramidal tract. Toxicity similar to that reported for TCP, has been found with cresyl diphenyl phosphate (Santicizer 140).

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Lefaux (1) in his book, Practical Toxicology of Plastics, cites TCP as being the most dangerous, not only of the phosphate esters, but of all of the plasticizers in general use. He also cautions concerning the indiscriminate use of cresyl diphenyl phosphate.

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B. Review of Toxicological Results for Specific Phosphate Esters

Santicizer 141

Santicizer 141 is regulated by the FDA Bureau of Foods under:

- 121.2514 Resinous and Polymeric Coatings
- 121.2520 Adhesives
- 121.2526 Resinous and Polymeric Coatings for Paper and Paperboard
- 121.2569 Resinous and Polymeric Coatings for Polyolefin Films

This broad FDA coverage was a result of two-year chronic oral feeding studies on both rats and dogs run prior to 1960. At that time, the safety requirements for FDA regulation were not as stringent or as sophisticated as they are today. Under today's philosophy, FDA regulation for S-141 would probably be much more difficult.

Recently, some specific uses for S-141 have encountered problems. Kimberly-Clark found S-141 to cause dermatological reactions on humans when it was used in a PVC non-woven fabric binder. This was confirmed by our own studies. KC switched to di-2-ethylhexyl phthalate. Borden, after finding the acute intraperitoneal toxicity to be relatively high, replaced S-141 in a medical tubing formulation with Santicizer 334F.

In addition to the skin sensitivity problem, S-141 was ound to be moderately toxic to fish according to the judgment of our Medical Department. They also have found that S-141 does not cause demyelination and does not present a vapor hazard when used under normal conditions in PVC compositions.

Santicizer 148

No attempt has been made to obtain FDA regulation for S-148. Its' toxicology appears to parallel that of

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Santicizer 141. It has a low acute oral toxicity (LD_{50}). Indications of skin reactions exist. For some reason S-148 showed a lower order of fish toxicity than S-141. It does not exhibit demyelination and presents no vapor hazard. According to our Medical Department, no impediments stand in its way for use as a flame retardant in automotive upholstery and other uses.

Santicizer 140

Although the acute oral toxicity (LD_{50}) of S-140 is low, the product appears to be highly toxic to fish (more toxic to fish than TCP) and does cause demyelination--degeneration of the nerve sheath (myelin), causing neuro-toxicity. Demyelination occurred with S-140 prepared from both "high ortho" Merichem cresylic acid as well as S-140 prepared from "low ortho" Pitt-Consul cresylic acid. These data are shown in Table 1.

Santicizer 140 has been shown to inhibit cholinesterase activity in rabbits when absorbed through the skin. These results are in the APPENDIX (Exhibit 2).

No differences in fish toxicity were noted between the two levels of cresylic acid. Skin sensitization was shown to be negative and no vapor hazard is expected from the use of S-140 according to our Medical Department. Work to determine whether S-140 inhibits cholinesterase production via repeated skin contact is underway. Inhibition of cholinesterase formation also causes neurological problems.

TCP

The literature contains numerous studies (both animal and human) showing serious neurotoxicity of TCP. Most of the studies show the tri-o-cresyl phosphate isomer to cause the problems. The effects were noted from misuse of TCP per se as well as TCP from plastic applications. Our data from the Medical Department show it to

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Table 1

PHOSPHATE ESTER TOXICOLOGY

EFFECT OF ORTHO ISOMER CONTENT
ON
DEMYELINATION AND FISH TOXICITY

	Toxicity	
	Demyelination	Fish Toxicity
S-140 (hi ortho)[1]	positive	high
S-140 (low ortho)[2]	positive	high
TCP (hi ortho)[3]	positive	moderate
TCP (low ortho)[4]	positive	moderate
Kronitex 100[5]	positive	moderate-high

1 S-140 hi-ortho (Merichem) 0.3% o-cresol
1.8% o-ethyl phenol

2 S-140 low-ortho (Pitt-Consul) < 0.1% o-cresol (None detected)
< 0.1% o-ethyl phenol (None detected)

3 TCP hi-ortho (Merichem) 0.4% o-cresol
3.2% o-ethyl phenol

4 TCP low ortho (Pitt-Consul) 0.2% o-cresol
0.6 ~~3.2%~~ o-ethyl phenol

5 Kronitex 100 32% o-isopropyl phenol
14% m/p isopropyl phenol
46% phenol
8% diisopropyl phenol

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cause demyelination regardless of the cresylic acid source (Pitt-Consul or Merichem). Both TCP samples showed fish toxicity. The acute LD_{50} and other properties such as eye irration, skin sensitivity and vapor hazard appear to be low.

Triphenyl Phosphate

The Medical Department information shows TPP not to cause demyelination. It is highly toxic to fish according to the test procedure used. All other tests, including acute LD_{50}, indicate TPP to be essentially non-toxic.

Kronitex 100

This competitive product (~~tri~~ iso propyl diphenyl phosphate) also causes demyelination and is highly toxic to fish according to our data. The results of the other tests parallel that of TCP and S-140.

A summary of the toxicity data on the phosphate esters are in Table 2.

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Table 2

SUMMARY OF TOXICOLOGICAL DATA FOR PHOSPHATE ESTERS

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Compound	Rat Oral LD_{50} (mg/kg)	Rabbit Dermal LD_{50} (mg/kg)	Skin Irr.[1]	Eye Irr.[2]	Vapor Hazard	Demyelination	Fish Toxicity[3]
S-140[4]	10,400	5,010	0	8.0	-	positive	high
S-141	15,800	7,940	2.6	12.6	nil	negative	moderate
S-148	15,800	7,940	3.0	10.6	nil	negative	low
TCP	15,800	2,000	0.6	9.3	nil	positive	moderate
TPP	10,800	7,940	0	6.0	-	negative	mod-high
Kronitex 100	10,000	5,010	0	10.0	nil	positive	mod-high
P-2XC-20	2,250	7,940	0	8.6	nil(non-vol)	-	-
P-1227	1,560	7,940	0	6.6	nil(non-vol)	underway	low

[1] Maximum score = 8

[2] Maximum score = 110

[3] Low = not very toxic
High = fairly toxic
Mod-high = split effect on 2 species

[4] S-140 inhibits cholinesterase activity upon repeated skin contact

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VI. REFERENCES

(1) Lefaux, Rene, PRACTICAL TOXICOLOGY OF PLASTICS, English Edition, CRC Press, 1968.

(2) Raizman, Paula, Literature Search on Neurotoxicity of Triaryl Phosphates, Report No. 3754, Job Number 1001-L-72-1, March, 1972.

(3) Levinskas, G. J., Phosphate Ester Plasticizer Toxicity Report, May 11, 1973

(4) Industrial Biotest Laboratories, Subacute Blood Cholinesterase Activity Study with Santicizer 140 in Albino Rabbits, BTL-72-98, April 13, 1973.

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Monsanto

FROM (NAME & LOCATION) Medical, A2SC

DATE May 11, 1973 cc R. W. Bucknell - B3NB

SUBJECT Phosphate Ester Plasticizers

REFERENCE

TO : P. R. Graham
B3NB

The table and attachments summarize available toxicity data on these products and provides an overall assessment of the findings. The terms behind the symbols in the table are broad. Acceptable means that no unique problems are to be expected in routine use of the material. Unfavorable signifies that special precautions may be needed in some instances. Questionable, at present, indicates that the status of the information is uncertain.

After you have had an opportunity to review this material, I would like to get together with you to discuss our next steps regarding the ongoing evaluation of safety of these materials.

George J. Levinskas

/bks

att.

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IN - 10 REV. 1/72

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Test	Product					
	S-140	S-141	S-148	TCP	TPP	Kronitex 100
Acute toxicity, irritation	A	A	A	A	A	A
Demyelination	U	A	A	U	A	U
Acute fish toxicity	U	A	A	A	U	A
Biodegradability	A	A	Q	Q	Q	Q
Sensitization	-	A	A	-	-	-

A=Acceptable
Q=Questionable
U=Unfavorable

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5/11/73

S-140
(high ortho content)
(cresyl diphenyl phosphate)

Acute toxicity screen

Report Y-72-82

Rat oral LD_{50} - 8250 mg/kg
Rabbit dermal MLD - greater than 1260 mg/kg
less than 2000 mg/kg
Irritation-rabbit skin - non-irritating(0.0/8)
-rabbit eye - slight (9.3/110)

Demyelination

Report BTL-71-66

Hen oral LD_{50} - greater than 10 g/kg
dosed 10 g/kg twice daily for 3 days;
showed gross signs of neurotoxicity
and demyelination

Acute fish toxicity

Report BTL-71-66A

Bluegills - TL_{50} greater than 0.1 ppm
less than 1.0 ppm
Trout - TL_{50} - greater than 0.1 ppm
less than 1.0 ppm

high ortho content=lot 188743#4

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5/11/73

S-140
(low ortho content)
(cresyl diphenyl phosphate)

Acute toxicity screen

Report Y-72-87	Rat oral LD_{50} 10,400 mg/kg Rabbit dermal MLD - greater than 5010 mg/kg less than 7940 mg/kg Irritation-rabbit skin - non-irritating (0.0/8) -rabbit eye - slight (8.0/110)
Report BTL-72-98	Rabbit dermal - single dose of 6176 mg/kg; no appreciable difference in susceptibility of male and female animals.

Demyelination

Report BTL-71-65	Hen oral LD_{50}- greater than 10 g/kg dosed 10 g/kg twice daily for 3 days; showed gross signs of neurotoxicity and demyelination

Acute fish toxicity

Report BTL-71-65A	Bluegills - TL_{50} - greater than 1.0 ppm less than 10.0 ppm Trout - TL_{50} - greater than 0.1 ppm less than 1.0 ppm

low ortho content=lot 188743#3

Biodegradation

Memo EST to JFQ, 4/5/73	SCAS Test. 82±12% disappearance at 5 mg feed rate. No inhibition of sludge growth.

Subacute Rabbit dermal study

Report BTL-72-98 (IBT No. A-2564, acute) (IBT No. 601-02564-subacute)	Dermal LD_{50}= 6176 mg/kg. 5M and 5F rabbits per group. Dosed 1/5 and 1/10 LD_{50} per day for 15 days. 10/10 deaths at high level, 7/10 at lower level. Mild skin irritation. Body weight loss. Inhibition plasma and RBC cholinesterase.

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S-141
(2-ethylhexyl diphenyl phosphate)

Acute toxicity screen

Report Y-71-121 — Rat oral MLD - greater than 15,800 mg/kg
Rabbit dermal MLD - greater than 7940 mg/kg
Irritation-rabbit skin - mild (2.6/8)
-rabbit eye - slight(12.6/110)

Demyelination

Report BTL-72-72 — Hen oral LD_{50} - greater than 10 g/kg
Dosed 10 g/kg twice daily for 3 days; repeated dose schedule 21 days later; neither gross nor microscopic signs of demyelination.

Acute fish toxicity

Report IBT No. A1820 — Bluegills TL_{50} = greater than 10.0 ppm less than 100 ppm

Trout TL_{50} = greater than 1.0 ppm less than 10.0 ppm

Human sensitization

Report SH-68-5 — Two-patch test on 200 subjects. Paper with S-141. Moderately severe primary irritant but not a sensitizer. 30/200 showed irritation.

Biodegradation

Memo EST to JFQ, (4/5/73) — SCAS Test. 74±9% disappearance at 5 mg feed rate. No inhibition of sludge growth.

Repeated feeding studies

AMA Arch Ind Hyg & Occ Med 8, 170-184 (1953)

Rats. 20M and 20F per group. Diets containing 5%, 1%, 0.125%,0.0625% fed for 2 yrs. 5% group died in a few wks. because of inanition. Growth retarded at 1% level. No significant effects on mortality. No relevant pathology.

Dogs. 2 dogs fiven 0.5 mg/kg/day. 6 days/wk for 2 yrs. 2 other dogs 1.0 ml on same schedule. Growth retarded in 1 ml/kg/day group (ca. 2.5% in diet). No deaths. No relevant pathology.

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S-141
(2-ethylhexyl diphenyl phosphate)

(continued)...

Metabolism

AMA Arch. Ind Hyg & Occ Med 8, 268-280 (1953	Rabbits. Repeated oral doses. Average 94% of phenol recovered in urine & feces. Essentially all fecal material parent compound. Urine, 14% parent compound, nearly 7% free or conjugated phenol. Man. Repeated oral doses. Average 96% of phenol recovered in urine & feces. Essentially all fecal material parent compound. Urine, 8% parent compound, nearly 7% free of conjugated phenol.

Skin sensitization

AMA Arch Ind Hyg & Occ Med 8, 284, 285 (1953)	Films. 2 parts PVC + 1 part S-141; 2 parts PVC + ½ part S-141 and ½ part DOP. 200 subjects. 5-day contact first patch. Challenge 10 or more days after removal. Neither an irritant nor a sensitizer.

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(5/11/73)

S-148
(isodecyl diphenyl phosphate)

Acute toxicity screen

Report Y-71-120	Rat oral MLD - greater than 15,800 mg/kg Rabbit dermal MLD - greater than 7940 mg/kg Irritation-rabbit skin - mild (3.0/8) -rabbit eye - slight (10.6/110)

Demyelination

Report BTL-72-71	Hen oral LD_{50} - greater than 10 g/kg dosed 10 g/kg twice daily for 3 days; repeated dose schedule 21 days later; neither gross nor microscopic signs of demyelination (1 bird died).

Acute fish toxicity

Report IBT No. 1820	Bluegills TL_{50} = greater than 10.0 ppm Trout TL_{50} = greater than 10.0 ppm less than 100 ppm

Human Sensitization

Report SH-68-5	Two-patch test on 200 subjects. Paper with S-148. Neither a primary irritant nor a sensitizer.
Report SH-68-9	15 applications (Mon., Wed., Fri. for 5 weeks with rest periods on Tues., Thurs., Sat., & Sun.). 50 subjects. Plastic film with S-148. Neither an irritant nor a fatiguing agent nor a sensitizer. As above. 50 subjects. 01/ml S-148 per application. Neither an irritant nor a fatiguing agent nor a sensitizer.

Biodegradation

Memo EST to JFQ 4/5/73	SCAS Test. 54±6% disappearance at 5 mg feed level. 17% disappearance at 20 mg feed level. Inhibition of sludge growth at both feed levels.

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(5/11/73)

TPP
(Triphenyl Phosphate)

Acute toxicity screen

Report Y-72-88 — Rat oral LD_{50} - 10,800 mg/kg
Rabbit dermal MLD - greater than 7940 mg/kg
Irritation - rabbit skin - non-irritating(0.0/8)
- rabbit eye - slight (6.0/110)

Demyelination

Report BTL-72-74 — Hen oral LD_{50} - greater than 5.0 g/kg
Dosed 5 g/kg twice daily for 3 days; repeated dose schedule 21 days later; neither gross nor histological signs of demyelination

Acute fish toxicity

Report IBT No. A1820 — Bluegills TL_{50} - greater than 1.0 ppm less than 10.0 ppm

Trout TL_{50} = greater than 0.1 ppm less than 1.0 ppm

Biodegradation

Memo EST to JFQ 4/5/73 — SCAS. 96±2% disappearance at 5 mg feed level. 93±11% disappearance at 20 mg feed level. No inhibition of sludge growth at 5 mg level. Inhibition at 20 mg level.

Animal toxicity and workroom assessment

AMA Arch. Environ Health, 1,33-46(1960)

Animals. Overall, low acute toxicity, 2.6 cats given 0.2g/kg and 0.4 g/kg IP showed general illness and paralysis 16-18 days later.

Oral and IP doses in mice produced slight lowering of whole blood cholinesterase. Dermal doses in ethanol did not.

Groups of 5 rats each fed 0.1% and 0.5% of TPP for 35 days led to growth depression & increased liver weights in the 0.5% group. No effect on hematology. No gross abnormalities.

Human. No evidence adverse clinical effects in man exposed for as long as (0 year to TPP vapor, mist and dust at a weighted average air conc. of 3.5 mg/m^3.

Threshold Limit Value (1973) 3 mg/m^3

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5/11/73

TCP
(high ortho content)

Acute toxicity screen

Report Y-72-81

Rat oral MLD - greater than 15,800 mg/kg
Rabbit dermal MLD - greater than 1260 mg/kg
less than 2000 mg/kg
Irritation - rabbit skin - non-irritating (0.0/8)
- rabbit eye - slight (10.0/110)

Demyelination

Report BTL-71-64

Hen oral LD_{50} - greater than 10 g/kg
dosed 10 g/kg twice daily for 3 days;
showed gross signs of neurotoxicity and
demyelination

Acute fish toxicity

Report BTL-71-64A

Bluegills - TL_{50} - 10.0 ppm
Trout - TL_{50} - greater than 10.0 ppm
less than 100 ppm

high ortho content=lot 188743#2

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TOWOLDMON0056301

5/11/73

TCP
(low ortho content)
(Tri-o-cresyl phosphate)

Acute toxicity screen

Report Y-72-80	Rat oral MLD - greater than 15,800 mg/kg Rabbit dermal MLD - greater than 2000 mg/kg less than 3160 mg/kg Irritation - rabbit skin - non-irritating (0.6/8) - rabbit eye - slight (9.3/110)

Demyelination

Report BTL-71-63	Hen oral LD_{50} greater than 10 g/kg dosed 10 g/kg twice daily for 3 days; showed gross signs of neurotoxicity and demyelination

Acute fish toxicity

Report BTL-71-63A	Bluegills - TL_{50} - greater than 1.0 ppm Trout - TL_{50} - greater than 1.0 ppm less than 10.0 ppm

low ortho content = lot 188743#1

Biodegradation

Memo EST to JFQ, 4/5173	SCAS test, 97+% disappearance at 5 mg feed level, 99+% disappearance at 20 mg feed level. No inhibition of sludge growth at 5 mg level. Inhibition at 20 mg level.

Threshold Limit Value (1973) 0.1 mg/m^3

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TOWOLDMON0056302

5/11/73

KRONITEX 100

(isopropyl phenyl diphenyl phosphate)

Acute toxicity screen

Report Y-72-111

Rat oral MLD - greater than 10,000 mg/kg
less than 12,600 mg/kg
Rabbit dermal MLD - greater than 5010 mg/kg
less than 7940 mg/kg
Irritation-rabbit skin - non-irritating(0.0/8)
-rabbit eye - slight(10.0/110)

Demyelination

Report BTL-72-46 and BTL-72-73

Hen oral LD_{50} - greater than 10 g/kg
Dosed 10 g/kg twice daily for 3 days; repeated dose schedule 21 days later; showed gross signs of neurotoxicity and histological evidence of demyelination.

Acute fish toxicity

Report BTL-72-47

Bluegills - TL_{50} = greater than 1.0 ppm
less than 10.0 ppm

Trout - TL_{50} = greater than 1.0 ppm
less than 10.0 ppm

Biodegradation

Memo EST to JFQ, 4/5/73

SCAS test. 49±8% disappearance at 5 mg feed level, 43% disappearance at 20 mg feed level. No inhibition of sludge growth at 5 mg level. Inhibition at 20 mg level.

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Exhibit 2

Santicizer 140 Cholinesterase Study

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Industrial **BIO-TEST** *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

REPORT TO

MONSANTO COMPANY

SUBACUTE BLOOD CHOLINESTERASE ACTIVITY STUDY WITH S-140 IN ALBINO RABBITS

BTL-72-98

APRIL 13, 1973

IBT NO. 601-02564

TOWOLDMON0056305

Industrial **BIO-TEST** *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

April 13, 1973

Dr. P. L. Wright
Monsanto Company
800 N. Lindbergh Boulevard
St. Louis, Missouri 63166

Dear Dr. Wright:

Re: IBT No. 601-02564 - Subacute Blood Cholinesterase Activity Study with S-140 in Albino Rabbits - BTL-72-98

We are submitting herewith our laboratory report dated April 13, 1973, prepared in connection with the above study

Very truly yours,

J. C. Calandra
President

JCC/kjl

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TOWOLDMON0056306

REPORT TO

MONSANTO COMPANY

SUBACUTE BLOOD CHOLINESTERASE ACTIVITY STUDY WITH
S-140
IN ALBINO RABBITS

BTL-72-98

APRIL 13, 1973

IBT NO. 601-02564

I. Introduction

At the request of Monsanto Company, a subacute blood cholinesterase activity study was conducted with S-140.

The purpose of this study was to determine if S-140 by repeated dermal application inhibited cholinesterase activity in the plasma and red blood cell. Recovery time and cumulative effects were also studied.

An outline of the investigation is presented in Table I.

The material in this report is to be used in development of the product and may be given to responsible sales contacts, but it is not to be used by them in advertising copy. The source of this material is not to be divulged until it appears in formal publications. No exceptions to the established rule may be made without the approval of the Medical Department in St. Louis. Customers' inquiries regarding matters of toxicity are to be referred as before to the Medical Department in St. Louis for reply.

—Monsanto Company

TOWOLDMON0056307

TABLE I

TEST MATERIAL: S-140

Subacute Blood Cholinesterase Activity Study - Albino Rabbits

Outline of Investigation

Test Material	Group	Number of Animals Males	Number of Animals Females	Concentration of S-140	Dose Level of Test Material (ml/kg)	Dose Level of S-140 (mg/kg)	Number of Dermal Applications
Tap Water	Treated Control	5	5	-	1.0	-	20
S-140	T-I	5	5	Undiluted	0.518	617.6	20
S-140	T-II	5	5	Undiluted	1.036	1,235.2	20

Note: S-140 was allowed to contact the shaved, occluded skin for six hours per day. The test animals were dosed five days per week for a period of four weeks. The skin of the last two males and two females in each group was abraded.

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II. Summary

A subacute blood cholinesterase activity study was conducted with S-140 using albino rabbits as test animals. The results of this study were:

A. Mortality

Seven animals in group T-I and all animals in group T-II died. The deaths were associated with S-140 intoxication. In addition, three animals in the Treated Control Group died. These deaths were due to naturally occurring disease.

B. Reactions

Diarrhea was noted among half of the animals in group T-I and all animals in group T-II during the application period. The diarrhea subsided during the recovery period.

Most of the animals in group T-II were emaciated prior to death.

S-140 was mildly irritating to rabbit skin.

C. Body Weights

Significant losses in body weights were noted among animals in groups T-I and T-II.

D. Blood Cholinesterase Activity Determinations

Significant depressions in plasma and erythrocyte cholinesterase activity were noted among animals in groups T-I and T-II at test days 13 and 29 and recovery days 7, 14, and 27 (group T-I). By recovery day 41, plasma cholinesterase activity was normal and erythrocyte cholinesterase activity was almost normal.

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1. Plasma Cholinesterase Activity Data

Plasma Cholinesterase Activity Data Expressed As Percent of Pretest Baseline Activity

		Test Day:		Recovery Day:			
Group	Sex	13	29	7	14	27	41
Treated	M	107	126	125	122	128	119
Control	F	112	119	115	110	125	113
T-I	M	42	48	53	63	97	86
	F	41	51	47	48	70	89
T-II	M	30	-	-	-	-	-
	F	32	45	-	-	-	-

- = Animal died.

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2. Erythrocyte Cholinesterase Activity Data

Erythrocyte Cholinesterase Activity Data Expressed As Percent of Pretest Baseline Activity

		Test Day:		Recovery Day:			
Group	Sex	13	29	7	14	27	41
Treated	M	92	106	96	93	102	112
Control	F	100	109	95	100	105	126
T-I	M	39	42	36	40	62	103
	F	38	40	35	30	56	76
T-II	M	45	-	-	-	-	-
	F	38	40	-	-	-	-

- = Animal died.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by: William J. Hamilton
William J. Hamilton, B.S.
Assistant Toxicologist
Acute Toxicity

Report approved by: Carmen Mastri
Carmen Mastri, B.S.
Senior Group Leader
Acute Toxicity

M. L. Keplinger
M. L. Keplinger, Ph.D.
Manager, Toxicology

mcb

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III. Investigational Procedure

A. Experimental Animals

Young adult albino rabbits of the New Zealand strain ranging in body weight from two to three kilograms were used. A total of 30 rabbits was selected after examination of each animal for general health and suitability for the test. Each animal was housed individually in a wire-bottomed cage for the duration of the test. All rabbits were fed a standard laboratory ration and water ad libitum.

B. Method of Application

In preparation for the test, the back of each rabbit was shaved using electric clippers. The shaved area of each animal constituted approximately 30 percent of the total body surface area. The animals were then returned to their cages and 24 hours was allowed to elapse before the first dermal applications were made. The waiting period permitted the skin to recover from the slight disturbance of the stratum corneum caused by the clipping procedure and also permitted the healing of any microscopic abrasions possibly produced during the shaving procedure. The shaving procedure was repeated as needed.

At the end of the 24-hour recovery period, just prior to the first application, the shaved skin of the last two males and two females in each group was abraded by making a series of parallel epidermal incisions, every two or three centimeters longitudinally, over the area of exposure. These incisions were made sufficiently deep to penetrate the stratum corneum but not to disturb the derma. This abrading procedure was repeated weekly.

TOWOLDMON0056312

To prevent oral ingestion of the test materials, each animal was fitted with a lightweight, flexible plastic collar which was worn throughout the investigational period.

S-140 was evaluated undiluted at dose levels of 617.6 mg/kg and 1,235.2 mg/kg. The test material was gently distributed over the application site (≈10% of total body surface area) of each animal and immediately occluded with an impervious plastic wrap. After a contact period of six hours, the wrap was removed and the skin thoroughly rinsed with tap water and patted dry. These dosing procedures were followed five days per week for four weeks for a total of 20 applications. Dose calculations were made weekly to adjust for changes in body weight.

Animals in the Treated Control Group received 20 dermal applications of tap water and were treated in every other respect as the test animals.

All animals were held for a 42-day recovery period following the 28-day application period.

C. Mortality and Body Weights

Observations for mortality and symptoms of intoxication were made daily.

D. Body Weights

Each animal was weighed on test days 0, 7, 14, 21, and 28 and recovery days 7, 14, 21, 28, and 42. The data were recorded as an index of health.

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E. Blood Cholinesterase Activity Determinations

Blood samples were withdrawn from the orbital sinus of each animal prior to the start of the study, at test days 13 and 29, and at recovery days 7, 14, 27, and 41. Cholinesterase activities in the plasma and red blood cell were determined using the automated method of Levine et al.*

* Levine, J. B., Scheidt, R. A. and Nelson, V., "An Automated Micro-Determination of Serum Cholinesterase," Automation in Analytical Chemistry (Technicon Symposia), New York, 1965, pp. 582-585.

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IV. Results

A. Mortality

Individual mortality data are presented in Table II.

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TABLE II

TEST MATERIAL: S-140

Subacute Blood Cholinesterase Activity Study - Albino Rabbits

Mortality Data

Test Material	Group	Number Dead / Number Tested	Animal Number and Sex	Number of Applications Prior to Death	Days on Test
Tap Water	Treated Control	3/10	3-M	20	62
			4-M	20	39
			10-F	20	69
S-140 (617.6 mg/kg)	T-I	7/10	11-M	20	36
			12-M	20	58
			13-M	20	47
			14-M	20	47
			16-F	20	40
			18-F	20	42
			19-F	20	33
S-140 (1,235.2 mg/kg)	T-II	10/10	21-M	8	12
			22-M	18	25
			23-M	14	21
			24-M	13	19
			25-M	18	26
			26-F	7	8
			27-F	20	28
			28-F	20	33
			29-F	8	12
			30-F	18	26

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B. Reactions

Diarrhea was noted among one animal in group T-I and five animals in group T-II after four applications. After ten applications, five animals in group T-I and all animals in group T-II had diarrhea. The diarrhea persisted throughout the remainder of the 29-day application period and subsided during the recovery period.

Most of the animals in group T-II were emaciated prior to death.

The test material was mildly irritating to rabbit skin. Barely perceptible to pale red erythema and barely perceptible edema (edges of area not defined) were noted at the site of contact of S-140 (groups T-I and T-II) during the entire application period. These reactions subsided during the recovery period.

C. Body Weights

Individual and mean body weight data are presented in Tables III through V.

TOWOLDMON0056317

TABLE III

TEST MATERIAL: Tap Water

Subacute Blood Cholinesterase Activity Study - Albino Rabbits

Body Weight Data

Animal Number and Sex	Individual Body Weights (kilograms) Test Day Number: 0	7	14	21	28	28-Day Weight Gains	Recovery Day Number: 7	14	21	28	35	42
1-M	3.24	3.24	3.30	3.50	3.34	0.10	3.58	3.70	3.80	3.88	3.84	3.46
2-M	2.80	2.82	2.76	2.84	2.82	0.02	2.94	3.12	3.14	3.18	3.18	3.00
3-M	3.04	3.15	3.20	3.28	3.22	0.18	3.40	3.50	3.70	3.66	(3.08)	-
4-M*	3.18	3.37	3.50	3.70	3.76	0.58	3.88	(3.34)	-	-	-	-
5-M*	3.02	3.00	2.94	2.98	3.00	-0.02	3.22	3.40	3.50	3.56	3.58	3.62
Mean	3.06	3.12	3.14	3.26	3.23	0.17	3.40	3.43	3.54	3.57	3.53	3.36
6-F	3.50	3.54	3.30	3.30	3.34	-0.16	3.68	3.92	4.10	4.16	4.30	4.44
7-F	2.50	2.61	2.68	2.80	2.84	0.34	3.18	3.40	3.62	3.74	3.70	3.88
8-F	2.62	2.85	3.02	3.04	3.08	0.46	3.38	3.52	3.66	3.56	3.84	4.06
9-F*	3.30	3.63	3.76	3.80	3.50	0.20	3.68	3.88	3.96	3.90	4.10	4.14
10-F*	3.34	3.29	3.20	3.14	3.10	-0.24	3.28	3.46	3.42	3.30	3.36	(3.02)
Mean	3.05	3.18	3.19	3.22	3.17	0.12	3.44	3.64	3.75	3.73	3.86	4.13

Note: Figures in parentheses were the weights of postmortem animals and were not included in the means.

* Abraded skin

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TABLE IV

TEST MATERIAL: S-140 (617.6 mg/kg)

Subacute Blood Cholinesterase Activity Study - Albino Rabbits

Body Weight Data

Animal Number and Sex	Individual Body Weights (kilograms) Test Day Number: 0	7	14	21	28	28-Day Weight Gains	Recovery Day Number: 7	14	21	28	35	42
11-M	3.28	3.23	3.20	3.24	2.84	-0.44	(2.48)	-	-	-	-	-
12-M	2.90	2.78	2.74	2.80	2.70	-0.20	2.80	3.14	3.06	2.92	(2.72)	-
13-M	3.80	3.35	3.30	3.04	2.74	-1.06	2.70	2.54	(2.06)	-	-	-
14-M*	2.76	2.76	2.80	2.78	2.84	0.08	2.90	3.06	(2.54)	-	-	-
15-M*	2.40	2.37	2.40	2.50	2.40	0.00	2.68	2.94	3.04	3.14	3.20	3.36
Mean	3.03	2.90	2.89	2.87	2.70	-0.32	2.77	2.92	3.05	3.03	3.20	3.35
16-F	2.44	2.35	2.38	2.48	2.40	-0.04	2.54	(2.28)	-	-	-	-
17-F	2.90	3.03	2.94	2.50	2.80	-0.10	2.94	3.06	3.30	3.32	3.16	3.10
18-F	3.72	3.78	3.64	3.48	3.38	-0.34	3.64	(3.02)	-	-	-	-
19-F*	2.86	2.65	2.60	2.94	2.52	-0.34	(2.18)	-	-	-	-	-
20-F*	3.62	3.57	3.52	3.60	3.50	-0.12	3.64	3.86	4.02	3.98	4.06	4.20
Mean	3.11	3.08	3.02	2.80	2.92	-0.19	3.19	3.46	3.66	3.65	3.61	3.65

Note: Figures in parentheses were the weights of postmortem animals and were not included in the means.

* Abraded skin

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TABLE V

TEST MATERIAL: S-140 (1,235.2 mg/kg)

Subacute Blood Cholinesterase Activity Study - Albino Rabbits

Body Weight Data

Animal Number and Sex	Individual Body Weights (kilograms) Test Day Number: 0	7	14	21	28	28-Day Weight Gains	Recovery Day Number: 7	14	21	28	35	42
21-M	3.62	3.18	(2.72)	-	-	-	-	-	-	-	-	-
22-M	3.34	3.11	3.08	2.64	(2.12)	-	-	-	-	-	-	-
23-M	3.16	2.86	2.42	(2.10)	-	-	-	-	-	-	-	-
24-M*	2.60	2.38	1.84	(1.40)	-	-	-	-	-	-	-	-
25-M*	2.74	2.55	2.20	2.06	(1.60)	-	-	-	-	-	-	-
Mean	3.09	2.82	2.38	2.35	-	-	-	-	-	-	-	-
26-F	2.60	1.93	(1.80)	-	-	-	-	-	-	-	-	-
27-F	3.18	2.66	2.62	2.54	(2.10)	-	-	-	-	-	-	-
28-F	2.96	2.98	2.98	2.98	2.84	-0.12	(2.30)	-	-	-	-	-
29-F*	3.40	2.68	(2.20)	-	-	-	-	-	-	-	-	-
30-F*	2.82	2.58	2.26	1.98	(1.52)	-	-	-	-	-	-	-
Mean	2.99	2.57	2.62	2.50	2.84	-0.12	-	-	-	-	-	-

Note: Figures in parentheses were the weights of postmortem animals and were not included in the means.

* Abraded skin

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D. Blood Cholinesterase Activity Determinations

Individual and mean cholinesterase activity data for the plasma and red blood cell are presented in Tables VI through XI.

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TABLE VI

TEST MATERIAL: Tap Water

Subacute Blood Cholinesterase Activity Study - Albino Rabbits

Plasma Cholinesterase Data

Animal Number and Sex	Plasma Cholinesterase Activity - μ Mol SH/ml/3 minutes at 37 °C						
	Test Day Number:			Recovery Day Number:			
	-7	13	29	7	14	27	41
1-M	1.63	1.90	2.02	2.38	2.09	2.05	1.97
2-M	2.45	2.23	2.62	2.77	2.88	3.12	2.90
3-M	2.25	2.13	2.78	2.41	2.38	2.47	-
4-M*	1.63	1.96	2.14	2.09	-	-	-
5-M*	1.94	2.40	2.87	2.68	2.34	2.47	2.18
Mean	1.98	2.12	2.49	2.47	2.42	2.53	2.35
6-F	1.45	1.48	1.96	1.83	1.73	1.96	1.81
7-F	1.36	2.07	2.14	1.83	1.81	2.26	1.84
8-F	1.47	1.74	1.69	1.76	1.66	1.69	1.66
9-F*	2.25	2.13	1.85	2.06	1.86	2.26	2.15
10-F*	1.73	1.82	2.21	2.00	2.03	2.17	-
Mean	1.65	1.85	1.97	1.90	1.82	2.07	1.86

* Abraded skin

- = Animal died.

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TABLE VII

TEST MATERIAL: S-140 (617.6 mg/kg)

Subacute Blood Cholinesterase Activity Study - Albino Rabbits

Plasma Cholinesterase Data

Animal Number and Sex	Plasma Cholinesterase Activity - μ Mol SH/ml/3 minutes at 37°C Test Day Number: -7	13	29	Recovery Day Number: 7	14	27	41
11-M	2.25	1.10	1.07	1.05	-	-	-
12-M	1.73	0.80	0.96	1.05	1.45	2.26	-
13-M	1.43	0.49	0.55	0.62	0.77	-	-
14-M*	2.12	0.87	1.04	1.27	1.37	-	-
15-M*	2.00	0.77	0.94	1.07	1.22	1.46	1.64
Mean	1.91	0.81	0.91	1.01	1.20	1.86	1.64
16-F	1.63	0.90	0.96	1.03	-	-	-
17-F	1.63	0.71	0.83	0.77	1.07	1.46	1.64
18-F	2.18	0.79	1.11	0.84	-	-	-
19-F*	1.78	0.80	0.92	-	-	-	-
20-F*	1.86	0.60	0.82	0.75	0.68	1.09	1.61
Mean	1.82	0.76	0.93	0.85	0.88	1.28	1.62

* Abraded skin

- = Animal died.

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TABLE VIII

TEST MATERIAL: S-140 (1,235.2 mg/kg)

Subacute Blood Cholinesterase Activity Study - Albino Rabbits

Plasma Cholinesterase Data

Animal Number and Sex	Plasma Cholinesterase Activity - μ Mol SH/ml/3 minutes at 37° C Test Day Number: -7	13	29	Recovery Day Number: 7	14	27	41
21-M	2.42	-	-	-	-	-	-
22-M	1.94	0.48	-	-	-	-	-
23-M	1.49	0.74	-	-	-	-	-
24-M*	1.73	0.50	-	-	-	-	-
25-M*	2.81	0.74	-	-	-	-	-
Mean	2.08	0.62	-	-	-	-	-
26-F	1.89	-	-	-	-	-	-
27-F	1.94	0.85	0.92	-	-	-	-
28-F	1.73	0.54	0.77	-	-	-	-
29-F*	1.83	0.41	-	-	-	-	-
30-F*	1.86	-	-	-	-	-	-
Mean	1.85	0.60	0.84	-	-	-	-

* Abraded skin

- = Animal died.

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TABLE IX

TEST MATERIAL: Tap Water

Subacute Blood Cholinesterase Activity Study - Albino Rabbits

Erythrocyte Cholinesterase Data

Animal Number and Sex	Erythrocyte Cholinesterase Activity - μ Mol SH/ml/3 minutes at 37° C Test Day Number: -7	13	29	Recovery Day Number: 7	14	27	41
1-M	5.68	5.23	5.63	4.58	5.09	5.53	6.02
2-M	5.34	5.57	6.00	5.37	5.42	6.09	6.91
3-M	5.25	4.07	5.45	4.81	4.92	5.53	-
4-M*	6.23	5.60	6.89	6.63	-	-	-
5-M*	5.60	5.40	5.81	5.71	5.60	5.71	5.92
Mean	5.62	5.17	5.96	5.42	5.25	5.72	6.28
6-F	7.23	7.29	8.34	7.23	7.34	8.01	9.11
7-F	6.04	5.83	7.00	5.71	5.69	6.49	7.77
8-F	6.61	7.08	7.65	7.23	7.67	7.55	8.48
9-F*	5.34	5.15	5.18	4.58	4.92	5.17	6.11
10-F*	6.04	5.92	6.00	4.97	5.51	5.53	-
Mean	6.25	6.25	6.83	5.94	6.23	6.55	7.87

* Abraded skin

- = Animal died.

TOWOLDMON0056325

TABLE X

TEST MATERIAL: S-140 (617.6 mg/kg)

Subacute Blood Cholinesterase Activity Study - Albino Rabbits

Erythrocyte Cholinesterase Data

Animal Number and Sex	Erythrocyte Cholinesterase Activity - μ Mol SH/ml/3 minutes at 37° C						
	Test Day Number:			Recovery Day Number:			
	-7	13	29	7	14	27	41
11-M	4.85	2.44	2.52	1.68	-	-	-
12-M	6.42	2.38	2.45	2.35	3.08	3.24	-
13-M	6.13	2.21	2.59	2.23	2.13	-	-
14-M*	7.02	2.15	2.26	2.12	1.84	-	-
15-M*	5.17	2.27	2.65	2.29	2.31	4.16	6.11
Mean	5.92	2.29	2.49	2.13	2.34	3.70	6.11
16-F	6.61	2.27	2.39	2.12	-	-	-
17-F	6.61	2.15	2.32	1.90	1.90	4.16	4.95
18-F	5.68	2.32	2.32	2.01	-	-	-
19-F*	4.46	2.15	2.19	-	-	-	-
20-F*	5.42	2.15	2.45	2.06	1.50	2.33	3.84
Mean	5.76	2.21	2.33	2.02	1.70	3.24	4.40

* Abraded skin

- = Animal died.

TOWOLDMON0056326

TABLE XI

TEST MATERIAL: S-140 (1,235.2 mg/kg)

Subacute Blood Cholinesterase Activity Study - Albino Rabbits

Erythrocyte Cholinesterase Data

Animal Number and Sex	Erythrocyte Cholinesterase Activity - μ Mol SH/ml/3 minutes at 37° C						
	Test Day Number:			Recovery Day Number:			
	-7	13	29	7	14	27	41
21-M	4.85	-	-	-	-	-	-
22-M	3.67	2.09	-	-	-	-	-
23-M	6.04	2.21	-	-	-	-	-
24-M*	6.51	2.44	-	-	-	-	-
25-M*	4.24	2.38	-	-	-	-	-
Mean	5.06	2.28	-	-	-	-	-
26-F	7.23	-	-	-	-	-	-
27-F	7.23	2.15	2.26	-	-	-	-
28-F	5.86	2.21	2.26	-	-	-	-
29-F*	4.69	2.62	-	-	-	-	-
30-F*	5.60	-	-	-	-	-	-
Mean	6.12	2.33	2.26	-	-	-	-

* Abraded skin

- = Animal died.

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