I-57-5

YOUNGER LABORATORIES
Biochemists . . . Pharmacologists . . . Analysts

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# Certificate of Analysis

February 15th, 1957

SUBJECT -

Toxicological Investigation Of Santowax R.

Monsanto Sample No. 4 - Lot A-2008
Monsanto Project No. I-57-5

I-57-5

TEST CONDUCTED FOR -

Monsanto Chemical Company, St. Louis, Missouri

EXPERIMENTAL PROCEDURE -

A) Acute Oral Toxicity In Rats
The compound was warmed to its melting point of 36° to 42° Centigrade, and fed orally by stomach tube to Sprague-Dawley strain albino rats.
It was intended to find the [illegible] but no deaths occurred at dosages up to and including 10.0 grams per kilogram. A number of animals were fed Santowax R daily for three days to determine if a build-up in toxicity would develop. Observations were made for outward toxic symptoms.

The data are shown in Table I.

B) Toxicity By Skin Absorption In Rabbits
Liquified Santowax R was applied to the closely clipped, intact skin of New Zealand white rabbits and a rubber collar placed around the neck of each animal to prevent turning the head sufficiently to get at the sample. Observations were made for abnormal behavior, and since no deaths occurred, no autopsies were performed.

The results are given in Table II.

C) Skin Irritation In Rabbits
Undiluted Santowax R was applied to the clipped skin of albino rabbits and observations made over a period of several days for irritation.

The results, based on the scoring technique of Draize, Woodard and Calvery (J. of Pharm. and Exp. Therapeutics, Vol. 82, No. 4, Dec. 1944), are shown in Table III.

D) Eye Irritation In Rabbits
Santowax R, in the amount of 0.1 milliliter, was placed in the conjunctival sac of the right eye of albino rabbits and the resulting irritation scored according to Draize, Woodard and Calvery (J. of Pharm. and Exp. Therapeutics, Vol. 82, No. 4, Dec. 1944).

The data from several days observations are given in Table IV.

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[illegible] Fenwick [illegible] y,
St. Louis, [illegible]
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TABLE I

ACUTE ORAL TOXICITY OF 'SANTOWAX E'
FOR RATS

| Animal No. - Sex | Weight Gm. | Total Dose Gm./Kg. | Number Of Times Dosed | Fate |
|---|---|---|---|---|
| 1- Female | 195 | 0.5 | 1 | Survived |
| 2- Female | 215 | 1.0 | 1 | Survived |
| 3- Female | 190 | 2.0 | 1 | Survived |
| 4- Male | 230 | 3.0 | 1 | Survived |
| 5- Male | 205 | 4.0 | 1 | Survived |
| 6- Female | 185 | 5.0 | 1 | Survived |
| 7- Male | 200 | 7.5 | 1 | Survived |
| 8- Male | 235 | 10.0 | 1 | Survived |
| 9- Male | 220 | 15.0 | 1 | Survived |
| 10- Female | 190 | 20.0 | 1 | Survived |
| 11- Female | 210 | 30.0 | 1 | Survived |
| 12- Female | 195 | 40.0 | 1 | Survived |
| 13- Female | 250 | 50.0 | 1 | Survived |
| 14- Male | 245 | 50.0 | 1 | Survived |
| 15- Male | 255 | 50.0 | 2 | Survived |
| 16- Female | 185 | 50.0 | 2 | Survived |
| 17- Female | 200 | 60.0 | 3 | Survived |
| 18- Male | 215 | 60.0 | 3 | Survived |

DISCUSSION -

The highest single dose of 50.0 grams per kilogram was non-lethal. A multiple dose of 60.0 grams per kilogram, given over a three day period, was also non-lethal.

Severe diarrhea and temporary loss of appetite developed but this condition appeared to be [illegible] to [illegible] stomach. No real evidence of systemic toxicity was observed. After four to five days the animals appeared normal.

Santowax E was considered to be relatively innocuous when fed to rats.

TABLE II

TOXICITY OF 'SANTOWAX R' BY SKIN ABSORPTION
IN RABBITS

| Animal No. - Sex | Weight Kg. | Dose Gs./Kg. | Weight Change 5 Days After Dosing | Fate |
|---|---|---|---|---|
| 1 - Female | 1.6 | 2.0 | + 6.0 % | Survived |
| 2 - Male | 2.1 | 3.5 | + 5.0 % | Survived |
| 3 - Male | 1.9 | 5.0 | + 3.0 % | Survived |
| 4 - Female | 1.7 | 7.5 | + 4.5 % | Survived |
| 5 - Female | 1.6 | 10.0 | + 7.0 % | Survived |
| 6 - Female | 1.9 | 12.5 | + 5.0 % | Survived |

DISCUSSION -

The highest application of 12.5 grams per kilogram was non-lethal. Appetites remained normal and no indication of toxicity developed over an observation period of three weeks. All animals continued to gain weight.

Inasmuch as no change in activity was observed, Santowax R was classed as innocuous to rabbits by skin absorption.

To: [illegible]

Yo[illegible] [illegible] Certificate of [illegible] - [illegible] ([illegible])

TABLE III

SKIN IRRITATION IN [illegible] AFTER APPLICATION OF "[illegible]AX R"

| Animal No. | Numerical Evaluation At The End Of 2 Hours | 24 Hours | 48 Hours | 72 Hours |
|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 |
| Average | 0.0 | 0.0 | 0.0 | 0.0 |

DISCUSSION -

No observable erythema or edema developed over a seventy-two hour observation period. The animals showed no discomfort.

Santowax R was classed as non-irritating to intact rabbit skin.

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TABLE IV

EYE IRRITATION IN RABBITS AFTER APPLICATION OF "[illegible]AX R"

| Animal No. | Numerical Evaluation At The End Of 1 Hour | 24 Hours | 48 Hours | 72 Hours |
|---|---|---|---|---|
| 1 | 4 | 2 | 0 | 0 |
| 2 | 6 | 2 | 0 | 0 |
| 3 | 4 | 2 | 0 | 0 |
| Average | 4.6 | 2.0 | 0.0 | 0.0 |

DISCUSSION -

No discomfort was shown immediately after application. There was slight redness of the [illegible] conjunctivae with moderate lacrimation and no edema of consequence after [illegible] for an average score of 4.6 out of a possible 110.0. Iris and cornea were unaffected. [illegible] a trace of erythema was still present. Within forty-eight hours all animals received a score of zero.

Santowax R was classed as a very mild eye irritant.

To: Monsanto Chemical Company,
[illegible]
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SUMMARY -

Rats survived a single dose of Santowax R at a level of 50.0 grams per kilogram without any real evidence of systemic toxicity.

The compound was considered to be innocuous to rabbits by skin absorption. The highest application was 12.5 grams per kilogram.

Santowax R was non-irritating to intact rabbit skin.

Slight redness with moderate lacrimation and no edema was present after one hour when the compound was placed in the eyes of rabbits. An average score of 4.6 out of a possible 110.0 was recorded. Santowax R was classed as a very mild eye irritant.

YOUNGS LABORATORIES

Fred M. Younger

BY: FRED M. YOUNGER

[illegible]

[illegible] in St. Louis for reply.

— Monsanto Chemical Company

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