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NORTHBROOK, ILLINOIS
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February 11, 1963

Mr. Don G. Fowler, Project Manager
Lead Industries Association
292 Madison Avenue
New York 17, New York

Dear Mr. Fowler:

We are enclosing our proposal for range-finding toxicity tests on materials in which your association is interested. The cost of these tests will be $600.00 per material tested.

If you have any questions on these procedures please do not hesitate to call us. Thank you again for permitting us to submit our proposals.

Sincerely,

J. C. Calandra

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Director

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Encl.

RANGE-FINDING TOXICITY STUDIES

I. Range-Finding Acute Oral Toxicity - Albino Rats

A. Acute Oral Toxicity - Albino Rats

Healthy, young albino rats of the Sprague-Dawley strain with an average body weight of approximately 100 grams are used as test animals. The rats are divided into groups of four animals each for dosing purposes.

All animals used are kept under observation for five days prior to experimental use, during which period they are checked for general physical well-being and homogeneity. The animals are housed individually in stock cages and permitted a standard laboratory rat diet* plus water ad libitum until 16 hours immediately prior to oral intubation.

On the morning of the first test day, after a 16-hour fast (water permitted), the selected dose groups of four rats each are intubated with previously calculated doses of the test material. All doses are administered directly into the stomachs of the test rats using a hypodermic syringe equipped with a ball-pointed intubating needle.

* Rockland Rat Diet, Rockland Farms, New City, New York.

Following oral administration of the test material, the rats are returned to their stock cages and observed for the succeeding 14 days. All significant reactions and mortalities which occur are recorded. Particular attention is given to the observation during the first four hours after intubation.

At the end of the observation period, all data are collected and the range-finding acute oral mean lethal dose ($RFLD_{50}$) of the test material is calculated using the techniques of Weil*, Thompson**, and Thompson and Weil***.

* Weil, Carrol S.: Tables for Convenient Calculation of Median-Effective Dose (LD_{50} or ED_{50}) and Instructions in Their Use. Biometrics, Sept. 1952.

** Thompson, William R.: Use of Moving Averages and Interpolation to Estimate Median-Effective Dose. Bact. Rev., Nov. 1947.

*** Thompson, William R. and Weil, Carrol S.: On the Construction of Tables for Moving Average Interpolation, Biometrics, March 1952.

II. Range-Finding Acute Percutaneous Toxicity - Albino Rabbits

Young adult, New Zealand strain male albino rabbits are employed as test animals. The body weights of the rabbits used range from two to three kilograms. All rabbits are maintained under observation in the laboratory for five days prior to testing. During the pre-test period the animals are examined with respect to their general health and suitability as test animals. The rabbits are housed in stock cages and maintained on the standard laboratory rabbit ration*. Food and water are permitted ad libitum.

Twenty-four hours prior to the dermal applications, the backs of the rabbits are shaved free of hair with electric clippers. The animals are then returned to their stock cages to await testing on the following day. The 24-hour waiting period allowed recovery of the stratum corneum from the disturbance which accompanies the clipping procedure and also permits healing of any microscopic abrasions possibly produced during the process.

On the testing day, the dermal dose for each test rabbit is weighed accurately on an analytical balance. Applications of the test material are made in solid form on the exposure sites of the rabbits. Three dose levels employing four rabbits each are used. After each application, the exposure site is covered by wrapping the trunk of the animal with an

* Rockland Rabbit Diet, Rockland Farms, New City, New York

impervious plastic sheeting which is securely taped in place. This plastic wrap insures intimate contact of epidermis and test material.

The test material remains in contact with the skin for 24 hours. Behavioral reactions are observed and recorded during the contact period, after which the plastic sheeting is removed from each test rabbit. The exposure sites are examined for local reactions and the animals returned to their stock cages. At this time it will also be possible to obtain an estimate of the primary skin irritating properties of the test materials. Observations for mortality, local reactions, and behavioral abnormalities are continued for a total of 14 days following the skin applications.

At the end of the observation period, all data were collected and the range-finding acute percutaneous mean lethal dose ($RFLD_{50}$) of the test material is calculated using the techniques of Weil*, Thompson**, and Thompson and Weil***.

* See footnote *, page 2
** See footnote **, page 2
*** See footnote ***, page 2

III. Range-Finding Acute Inhalation Toxicity - Albino Rats

Young, adult albino rats of the Sprague-Dawley strain were employed as the test animals. Pre- and post-test care and observation of these rats are essentially the same as that already described for rats used in the range-finding acute oral toxicity test (see p. 1). However, animals used for inhalation exposure were not fasted prior to testing.

Five groups of four rats each are utilized in the study of the test material. The average body weight of the test animals are approximately 250 grams. The inhalation exposures are conducted in a multiple inhalation chamber designed for simultaneous exposure to atmosphere saturated and fractionally saturated with the test material, e.g. 1/2 saturated, 1/4 saturated, 1/8 saturated and 1/16 saturated at room temperature (25°C). The test is designed to run for a four-hour period.

At the end of the exposure period, the rats are observed for the succeeding 14 days.

IV. Range-Finding Eye Irritation Test - Albino Rabbits

A group of five albino rabbits are used to evaluate the eye irritating properties of the test material. Young adult, New Zealand strain animals are selected for the test.

The test method employed is patterned after that of Draize et al*. Exactly 50 mg of solid test material is instilled into the conjunctival sac of the right eye of each test rabbit in a group of five animals. The left eye of each animal serves as a scoring control.

One, 24, 48, 72, 96 hours, and 7 days following the initial instillations, the cornea, iris, and palpebral conjunctiva are examined individually and graded for irritation and injury according to a standard scoring system*. The maximum possible score at any one examination and scoring period is 110 points which indicates maximal irritation and damage to all three ocular tissues. Zero score indicates no irritation whatever.

After the completion of the test, the scores are analyzed and a descriptive eye irritation rating is assigned to the test material. The criteria used for assignment of the descriptive rating are the frequency, the extent, and the persistence of irritation or damage which occurred to the three ocular tissues. In the classification system used, special emphasis is placed upon irritation or damage to the cornea. Correspondingly less stress is placed upon conjunctival and iridial effects.

* "Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes", Draize, John H., Woodard Geoffrey, and Calvery, Herbert O., J. Pharm. & Exp. Ther. 82, 4, December 1946.

The descriptive rating assigned are one of the following, each of which characterizes a particular level of ophthalmic irritation and damage:

Non-Irritating
Practically Non-Irritating
Minimally Irritating
Mildly Irritating
Moderately Irritating
Severely Irritating
Extremely Irritating
Maximally Irritating

PILOT STUDY

Effect of lead acetate as a radioprotective agent

Materials and Methods

Wistar rats of both sexes weighing 100 gms. and on an ad libitum diet were used. Animals were divided into 6 groups as follows:

	100 mg/kg	200 mg/kg	250 mg/kg	
Group I	A (4)	B (4)	C (4)	NaCl
Group II	A (4)	B (4)	C (4)	$Pb(Ac)_2$
Group III	A (4)	B (4)	C (4)	$AgNO_3$
Animals	12	12	12	

Animals were injected with NaCl (negative control). $Pb(Ac)_2$ (experiment) or $AgNO_3$ (heavy metal control) in sterile distilled water. Route of administration was I. P. and doses were divided into 50 mg/kg equal doses. Following the last dose, 400 r of whole body X-irradiation was administered 24 hours later. Dose was corrected for skin level and no shielding of scatter was employed. All animals were returned to cages for routine care. At death, complete autopsy examinations were carried out including microscopic sections.

Results

Table I shows the death pattern following the X-ray exposure in all groups.

Days Post X-ray

		1	2	3	4	5	6	7	8
Group I	A	1	0	1	2	-	-	-	-
	B	1	1	0	2	-	-	-	-
	C	2	1	1	0	-	-	-	-
Group II	A	2	0	0	1	1	-	-	-
	B	1	0	0	0	1	1	0	1
	C	2	0	0	0	1	1	-	-
Group III	A	1	0	0	1	2	0	-	-
	B	0	0	0	0	1	2	1	-
	C	0	0	0	1	1	1	1	-
Total		10	2	2	7	6	5	3	1
Cumulative %		27.7	33.2	38.7	58.2	74.7	88.5	96.8	100
Expected %		30	-	-	-	70	-	-	100

Table II Pathological Observations

		Hemorrhages	Cytolysis	Bact. Inf.
Group I	A	++++	++++	+
	B	++++	++++	+
	C	++++	++++	+
Group II	A	+++	++++	+
	B	++	++++	+
	C	++	++++	±
Group III	A	++++	++++	+
	B	++++	++++	+
	C	++++	++++	+
Expected		++++	++++	+

Table I shows that the expected mortality rate was not altered by the agents administered in this study. However, Table II does indicate that the organ and serosal surface hemorrhages were significantly reduced in Group II B and C. Lymphocytolysis and bacteremia with colonization was not changed in these groups.

Conclusion

A pilot study to test the radioprotective action of inorganic ionized lead was carried out in Wistar rats. The cumulative mortality rate was not altered. However, the frequency and intensity of hemorrhages in organs and serosal surfaces in the lead acetate treated animals was less than in the controls. Further studies are indicated to more clearly evaluate this finding. The numbers of animals used and the LD 50 irradiation dose exposure should be modified in future research.

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