

Memorandum



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

010757

FEB 2 - 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: ID# 078003. SULFURYL FLUORIDE. Evaluation of Motor Activity Validation and Functional Observational Battery Proficiency Testing Studies Submitted by Dow Chemical Co. for Sulfuryl Fluoride Neurotoxicity Testing.

Tox. Chem. No.: 816A
PC No.: 078003
Submission No.: S447468
Barcode No.: D194799

FROM: Linnea J. Hansen, Ph.D. *Linnea J. Hansen* 1-21-94
Section IV, Tox. Branch I
Health Effects Division (H7509C)

TO: Larry Schnaubelt, Manager, PM Team 72
Robert Richards, Reviewer, PM Team 72
Special Review and Reregistration Division (H7508W)

THRU: Marion P. Copley, D.V.M., D.A.B.T., Section Head
Section IV, Tox. Branch I
Health Effects Division (H7509C) *Marion Copley* 1/25/94

CONCLUSIONS:

The motor activity validation study (MRID 428652-01) and the functional observational battery proficiency test (MRID 428652-02) were reviewed by TB-I and demonstrated sensitivity of the assays as performed by Dow's toxicology laboratory. These studies are considered adequate for satisfying demonstration of sensitivity of neurotoxicity testing methodology for sulfuryl fluoride.

Classification: Core-supplementary (supplemental information for 81-8 and 82-7).

The electrophysiological testing described in the submitted published article (90-day rat inhalation) is the same study that was previously reviewed in detail by TB-I (MRID 408399-02; HED Doc. no. 9479) and was therefore not reviewed here. The electrophysiological testing did not include positive controls to demonstrate technical proficiency or potential magnitudes of effects for comparison to the effects demonstrated in rats



Recycled/Recyclable
Printed with Soy/Canola Ink on paper that
contains at least 50% recycled fiber

010757

exposed to sulfuryl fluoride. However, at this time TB-I does not consider submission of a new validation study necessary for supporting neurotoxicity testing of sulfuryl fluoride since a NOEL and LOEL could be determined in this study.

ACTION REQUESTED:

On July 28, 1993, DowElanco submitted for review a validation study for motor activity testing in rats, a proficiency testing study in rats and an article entitled "Subchronic Neurotoxicity in Rats of the Structural Fumigant, Sulfuryl Fluoride" (Neurotoxicity and Teratology 10:127-133, 1988). These studies were submitted to fulfill requirements for neurotoxicity testing for sulfuryl fluoride by demonstrating ability of laboratory to conduct sensitive neurotoxicity testing.

010757

Primary Review: Linnea J. Hansen, Ph.D. *Linnea J. Hansen 1/26/94*
Review Section IV, Tox. Branch I
Secondary Review: Marion P. Copley, D.V.M., D.A.B.T.
Review Section IV, Tox. Branch I *Marion Copley 1/24/94*

DATA EVALUATION RECORD

STUDY TYPE: Neurotoxicity: Validation of Methodology (Rat)
Guideline: none (supplemental to 82-7 for sulfuryl fluoride)

MRID NO.: (1) 428652-01 (motor activity validation)
(2) 428652-02 (FOB proficiency study)

SPONSOR: Dow Chemical Company, Midland, Michigan

STUDY NOS.: (1) HET T1.05-018-002-REV (Motor Activity)
(2) HET T1.05-022-000-01 (FOB)

TESTING FACILITY: Toxicology Research Laboratory, Health and
Environmental Sciences, Dow Chemical Company,
Midland, Michigan

TITLE OF REPORTS: (1) Validation of a Motor Activity System for
Rats
(2) P.J. Spencer: Proficiency Demonstration
in Conduction of the Functional
Observational Battery

AUTHORS: (1) R.R. Albee, J.A. Pitt and J.L. Mattson
(2) R.R. Albee, P.J. Spencer and J.L. Mattson

REPORT ISSUED: (1) and (2) July 27, 1993

CONCLUSIONS:

Male Fischer 344 rats were examined following administration of pharmacologic agents causing effects on motor activity (d-amphetamine or chlorpromazine) and functional observational battery parameters (d-amphetamine, chlorpromazine or atropine/physostigmine), saline injection or no treatment. Motor activity validation and functional observational battery proficiency testing demonstrated ability of personnel to conduct neurotoxicity testing.

Classification: Supplementary

These studies were submitted in partial fulfillment of Neurotoxicity Guidelines requirements (validation of methodology) and are considered supplemental information to the neurotoxicity studies submitted on sulfuryl fluoride. The studies appeared to have been properly conducted and were considered acceptable for the intended regulatory purposes.

010757

Signed good laboratory practices and quality assurance statements were present.

MOTOR ACTIVITY VALIDATION STUDY

STUDY DESIGN: Male Fischer 344 rats were randomly assigned to the following test groups (Table 1) to test a pharmacologic agent (d-amphetamine) known to cause increased motor activity:

TABLE 1: ANIMAL ASSIGNMENT: D-AMPHETAMINE

Test Group	Dose Level (mg/kg)	Number Assigned males
Control	0	12
Saline-injected ¹	0.5 ML	12
Low Dose	0.1	12
Mid Dose	0.32	12
High Dose	1.0	12

¹ Saline administered in ml volume instead of mg/kg

One week later, the same group of 60 male Fischer 344 rats (12/dose group) used above were assigned in reverted order (eg control chlorpromazine = high dose amphetamine, etc.) to the following test groups (Table 2) to test a pharmacologic agent (chlorpromazine) known to cause decreased motor activity:

TABLE 2: ANIMAL ASSIGNMENT: CHLORPROMAZINE

Test Group	Dose Level (mg/kg)	Number Assigned males
Control	0	12
Saline-injected ¹	0.5 ML	12
Low Dose	0.5	12
Mid Dose	2.24	12
High Dose	5.0	12

¹ Saline administered in ml volume instead of mg/kg

All rats (except controls) were injected intraperitoneally in a total volume of 0.5 ml saline at doses based on body weight measured that day. Testing was initiated about 10 minutes after injection of first animal of each group. Animals were tested for 50 minutes (recorded in 10-minute intervals) in doughnut-shaped motor activity chambers equipped with a single infrared photocell beam intersecting the alley in 2 locations, 180° apart. Photocells were connected to counting and timing units to record beam breaks. A total of 5 chambers were operated simultaneously in a darkened room with no human entry during testing periods.

DATA EVALUATION: Motor activity data were transformed to their square roots to reduce heterogeneity of variance. Means and standard deviations were calculated and an F-max test for homogeneity of variance was performed, with statistical significance at $\alpha = 0.01$. Epoch square root data were analyzed using repeated measures univariate analysis of variance (rep-ANOVA), with statistical significance at $\alpha = 0.05$ and MANOVA to avoid sphericity of variance/covariance matrix of the rep-ANOVA.

4

010757

RESULTS/DISCUSSION: Results are summarized in Appendix I. A dose-dependent increase in motor activity was observed in rats treated with amphetamine (motor activity was increased above saline-injected controls by 17, 51 and 217% at 0.1, 0.32 and 1.0 mg/kg, respectively; statistically significant at 0.32 and 1.0 mg/kg). Rats treated with chlorpromazine showed decreases in total session motor activity (decreased below saline-injected controls by 28, 45 and 75% at 0.5, 2.24 and 5.0 mg/kg; statistically significant at 2.24 and 5.0 mg/kg). Saline-injected controls showed decreased total motor activity in both groups (23 and 12%; statistically significant). The mean total session activity of the 2 non-injected control groups varied less than 8% from each other. An asymptote was met during the last 20% of the test session for d-amphetamine and was approached for the chlorpromazine controls. The study therefore demonstrated sensitivity of the assay to pharmacologically-induced effects, positive and negative, and demonstrated that motor activity may be affected by non-neurotoxic agents (saline injection). Slightly longer test sessions may be required to ensure achievement of the asymptote in controls.

FUNCTIONAL OBSERVATIONAL BATTERY (FOB)

The FOB testing described in this report was the proficiency demonstration for Pamela J. Spencer.

STUDY DESIGN: Male Fischer 344 rats were randomly assigned to the following test groups (Table 3) to test pharmacologic agents known to affect specific parameters tested in the FOB:

TABLE 3: ANIMAL ASSIGNMENT

Test Group	Dose Level (mg/kg)	Number Assigned males
Saline-injected ¹	0.15 ML	5
Chlorpromazine HCl	4.0	5
D-Amphetamine sulfate	8.0	5
Atropine ²	2.0	5

¹ Saline administered in ml volume instead of mg/kg

² Atropine followed in 5 minutes with 0.75 mg/kg subcutaneous injection of physostigmine; examined between 10 - 15 min. post-injection

Each pharmacological agent was injected intraperitoneally in normal saline. FOB testing was initiated at the following times post-injection for animals of each test group: saline, 10-20 min.; chlorpromazine HCl, 15-20 min.; d-amphetamine sulfate, 15 min. and atropine plus physostigmine, 10-30 min. Animals could be reused for treatment with different agents after one week.

FOB proficiency training was conducted in 3 stages: (1) familiarity of technician with technology and mechanics of the FOB; (2) training in recognition of positive effects through coaching by trainer and (3) demonstration of proficiency in

conduct of testing and recognition of positive effects in animals treated with pharmacologic agents without coaching. The proficiency demonstration was conducted blindly with coded animals. Parameters observed in the FOB are listed in Appendix 2.

DATA EVALUATION AND EVALUATION OF OBSERVER PROFICIENCY:

Individual observations for each rat were presented in incidence summary tables with observations entered by category. Simplified composite summary tables were prepared for categorical observations by adding animals (1 animal = 1 point) for each observation category; for graded observations by adding the number of animals (points) with an observation multiplied by level points (1 - 5) indicating the severity of the observation; or for measurements, by presenting the group mean value for that measurement.

Composite scores were graphed as a diagram (complex waveform) for comparison to template complex waveforms from previously conducted FOBs. Scores were normalized to eliminate the baseline, or inherent magnitude of individual observations, so that correlation values (see below) would not be dominated by the baseline. Baselines were removed for each observation by averaging composite scores for each of the four treatments and subtracting the average from each of the four individual scores.

Results of proficiency testing were analyzed subjectively by the trainer to determine whether the expected pattern of observations for a particular pharmacologic agent was present. An objective evaluation of proficiency was conducted by calculation of a Pearson cross correlation coefficient between template scores reflecting expected syndromes and derived from scores of previous FOB testing by several technicians. A Pearson's r greater than 0.8 was considered indicative of a technician's ability to perform the FOB.

RESULTS/DISCUSSION: The proficiency testing for P.J. Spencer showed good correlation of observations with the template. The Pearson's r for categorical and ranked observations was 0.949 and for measurements of temperature, grip and splay was 0.959. A composite summary of the results compared to the template is shown in Appendix 3. Rats treated with d-amphetamine showed head weaving, rapid respiration, piloerection, salivation, pronounced extensor thrust, increased activity, response to touch and sharp noise and increased body temperature. Rats treated with chlorpromazine showed fixed postures, decreased activity and resistance to removal, decreased body temperature and increased forelimb grip and landing foot splay. Rats treated with atropine/physostigmine had tremors, dilated pupils, decreased muscle tone, activity, extensor thrust, tailpinch response and grip strength, and increased noise response and gait abnormalities (but not landing foot splay).

VIRANE (Sulfuryl fluoride)

TD Review 010759

Page _____ is not included in this copy.

Pages 7 through 10 are not included.

The material not included contains the following type of information:

- ____ Identity of product inert ingredients.
- ____ Identity of product impurities.
- ____ Description of the product manufacturing process.
- ____ Description of quality control procedures.
- ____ Identity of the source of product ingredients.
- ____ Sales or other commercial/financial information.
- ____ A draft product label.
- ____ The product confidential statement of formula.
- ____ Information about a pending registration action.
- ____ FIFRA registration data.
- ____ The document is a duplicate of page(s) _____.
- ____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.