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

CASWELL FILE 007756

FEB - 9 1990

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TOX Chem No.: 467
TB Project No.: 9-0441
RD Record No.: 235,444

MEMORANDUM

SUBJECT: GIBBERELLINS A4A7 (GA4A7) - Battery of Acute Study Data Submitted in Response to Biorational Testing Guidelines 152-10 Through 152-15 Tier Requirements

FROM: Irving Mauer, Ph.D., Geneticist 01/03/90
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

TO: T. Myers/B. Briscoe, PM Team 50
Reregistration Division (H7508C)

THRU: Karl P. Baetcke, Ph.D., Chief 1/13/90
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

Registrant: Abbott Laboratories, North Chicago, IL

Request

Review and evaluate a battery of six (6) acute studies with GA4A7 (90%)*, submitted to satisfy data requirements under FIFRA sections 135.152-10 through -15.

TB Conclusions

Summarized below, and also see attached detailed reviews.

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*All performed at Hazleton Labs America (HLA), Kensington, MD.

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Study Type/No. (Guideline)	Title (MRID No.)	Reported Results	TB Evaluation (TOX CAT.)
1. Acute Oral - Rat HLA 80602323 (152-10)	Acute Oral Toxicity Study With Gibberellins A4A7 (G A4A7) in Rats (40873201)	LD_{50} 5000 mg/kg (males/females)	Guidelines (IV)
2. Acute Dermal - Rabbit HLA 80602324 (152-11)	Acute Dermal Toxicity Study With Gibberellins A4A7 (G A4A7) in Rabbits (40873202)	LD_{50} 2000 mg/kg (males/females)	Guidelines (III)
3. Acute Inhalation - Rat HLA 375-141 (152-12)	Acute Inhalation Study With Gibberellins A4A7 (G A4A7) in Rats (40873203)	No lethality at 2.98 mg/L, reportedly the maximum TWA (actual) exposure level attainable	[See comments below*] (III)
4. Primary Eye Irritation - Rabbit HLA 80692326 (152-13)	Primary Eye Irritation Study With Gibberellins A4A7 (G A4A7) in Rabbits (40873204)	PEIS ranged from 19.3 at 1 hour, down to 4.7 at 96 hours; = 0.0 by 7 days.	Guidelines (III)
5. Primary Dermal Irritation - Rabbit HLA 80602325 (152-14)	Primary Dermal Irritation Study With Gibberellins A4A7 (G A4A7) in Rabbits (40873205)	PDIS = 0.0	Guidelines (IV)
6. Skin Sensitization - Guinea Pig HLA 80602327 (152-15)	Dermal Sensitization Study With Gibberellins A4A7 (G A4A7) in Guinea Pigs (40873206)	Mild Sensitizer	Minimum (III)

ATTACHMENTS 2

(*)

[Additional Comments by EPA Reviewer

Although the Dynamac reviewer classified this study as "Minimum," a Core Grade is not being assigned to this acute inhalation assay because of substantive problems in achieving essential criteria of assay acceptance according to the Agency's test guidelines, when using the test material as applied (a white powder, 90% ai), difficulties acknowledged by all concerned in the appraisal of the study (see below). Since no deaths occured at the highest dose reported as attainable (2.98 mg/L), however, we can agree that the Toxicity Category is no worse than III.

1. The Dynamac review pointed out the failure of the study investigator/reporter (J.B. Terrill, Hazleton) to: a) Address the uncertainty that a particle size (MMAD) of 5.8 microns could be inspirable and/or inhalable by the rat respiratory system; or b) provide estimates of the requisite percentage of particles that would be of inhalable size for this test species.

2. One Agency inhalation expert considered the study unacceptable because: a) Particle size was "too large to be either inhalable or inspirable," and the study author failed to justify or explain why they could not achieve a one-micron size; b) the exposure chamber was inadequately described ("not standard"); and c) no justification or rationale was given for the calculated gravimetric (actual) concentration of 2.98 mg/L ("the maximum attainable concentration," according to the study author).

3. The study investigator subsequently submitted additional information in defense of his position on the maximally achievable test substance concentration and particle size, consisting of: a) A published article describing the design, construction and operation of a dynamic inhalation exposure chamber and controls; and b) an agenda and reports from a recent (August 10, 1989) meeting of other (outside) inhalation experts (attached here) (*) addressing two critical issues, namely, the Agency's requirements that a limit test concentration be 5 mg/L, and the 1-micron particle size, as mandated by recent (inhouse) interpretations of FIFRA Test Guidelines for this type of assay.

(*) ATTACHMENT - I

4. Finally, the most recent FIFRA Test Guidelines for Acute Inhalation (OPP, November, 1984) state only that particle size for test compounds (TGAI, MP, or EP) be inhalable for man, i.e., aerodynamic diameters of 15 micrometers or less; and that a limit test can be first performed, at "an exposure of 5 mg/L (actual concentration of respirable substance) for 4 hours," or, "where this is not possible due to physical or chemical properties of the test substance, [at] the maximum attainable concentration . . . ."]

Attachments (DERs) - 11

EPA: 68D80056
DYNAMAC No.: 211-D
TASK No.: 2-11D
October 2, 1989

DATA EVALUATION RECORD

GIBBERELLINS

Acute Oral Toxicity Study in Rats

STUDY IDENTIFICATION: Glaza, S. M. Acute oral toxicity study of gibberellins A4A7 (GA4A7) in rats. (Unpublished study No. 80602323 conducted by Hazleton Laboratories America, Inc., Madison, WI, for Abbott Laboratories, North Chicago, IL; dated August 29, 1988.) Accession No./MRID No. 408732-01.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 10/2/89

1. CHEMICAL: Gibberellins A4A7 (GA4A7); gibberellic acid (GA3); 2,4a,7-trihydroxy-1-methyl-8-methylenegibb-3-ene-1,10-dicarboxylic acid 1,4-lactone.

2. TEST MATERIAL: Gibberellins A4A7 (GA4A7), Code 33691, lot No. 16-213-CD, contained approximately 90% active ingredient (48% Gibberellin A4 and 42% Gibberellin A7 by weight) and was described as a white powder.

3. STUDY/ACTION TYPE: Acute oral toxicity in rats.

4. STUDY IDENTIFICATION: Glaza, S. M. Acute oral toxicity study of gibberellins A4A7 (GA4A7) in rats. (Unpublished study No. 80602323 conducted by Hazleton Laboratories America, Inc., Madison, WI, for Abbott Laboratories, North Chicago, IL; dated August 29, 1988.) Accession No./MRID No. 408732-01.

5. REVIEWED BY:

Linda Plankenhorn, B.A.
Principal Reviewer
Dynamac Corporation

Signature: Linda J. Plankenhorn
Date: October 2, 1989

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: October 2, 1989

6. APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: Roman J. Pienta
Date: Oct 2, 1989

Irving Mauer, Ph.D.
EPA Reviewer, Insecticide/
Rodenticide Support
Toxicology Branch I
Health Effects Division
(H-7509C)

Signature:
Date: 10/02/89

Karl P. Baetcke Ph.D,
Chief, Insecticide/
Rodenticide Support
Toxicology Branch I
Health Effects Division
(H-7509C)

Signature:
Date: 10/3/89

6

7. CONCLUSIONS:

CORE Classification: CORE Guideline.

LD_{50}: >5000 mg/kg for male and female rats.

Toxicity Category: IV.

8. SUMMARY:

Five male and five female albino rats [CRL:CD®(SD)BR], weighing from 204 to 248 g, received single oral gavage administrations of gibberellins A4A7 at a dose level of 5.0 g/kg. The rats were fasted overnight prior to dose administration.

The test material was mixed with distilled water to form a suspension at a concentration of 0.5 g/mL and was administered in a dosing volume of 10.0 mL/kg body weight (5.0 g/kg). Rats were observed for mortality and clinical signs at 1, 2.5, and 4 hours postdosing and daily thereafter for 14 days. Body weights were measured initially on day 0 and on days 7 and 14. At study termination, all rats were euthanized and subjected to gross necropsy examination.

No mortality or abnormal clinical observations were noted for animals treated at a level of 5.0 g/kg except for diarrhea, which was noted in three females on the day of dosing. No treatment-related gross pathologic findings were noted at necropsy. The study author concluded that the acute oral LD_{50} value of the test material was greater than 5000 mg/kg.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

The conduct and reporting of the study were adequate. A quality assurance statement was signed and dated August 30, 1988.

The acute oral LD_{50} value of the test material in male and female rats was reported to be greater than 5000 mg/kg. The test material belongs in Toxicity Category IV.

10. CBI APPENDIX:

Appendix A, Experimental Design, CBI pp. 21-24.

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APPENDIX A

Experimental Design
(CBI pp. 21-24)

Gibberellins toxicology review

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___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

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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.

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EPA: 68D80056
DYNAMAC No.: 211-E
TASK No.: 2-11E
October 2, 1989

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

DATA EVALUATION RECORD

GIBBERELLINS

Acute Dermal Toxicity Study in Rabbits

STUDY IDENTIFICATION: Glaza, S. M. Acute dermal toxicity study of gibberellins A4A7 (GA4A7) in rabbits. (Unpublished study No. HLA 80602324 conducted by Hazleton Laboratories America, Inc., Madison, WI for Abbott Laboratories, North Chicago, IL; dated August 29, 1988.) Accession/MRID No. 408732-02.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 10/2/89

13

1. CHEMICAL: Gibberellins A4A7 (GA4A7); gibberellic acid (GA3); 2,4a, 7-trihydroxy-1-methyl-8-methylenegibb-3-ene-1,10-dicarboxylic acid 1,4-lactone.

2. TEST MATERIAL: Gibberellins A4A7 (GA4A7), Code 33691, lot No. 16-213-CD, contained approximately 90% active ingredient (48% gibberellin A4 and 42% gibberellin A7 by weight) and was described as a white powder.

3. STUDY/ACTION TYPE: Acute dermal toxicity in rabbits.

4. STUDY IDENTIFICATION: Glaza, S. M. Acute dermal toxicity study of gibberellins A4A7 (GA4A7) in rabbits. (Unpublished study No. HLA 80602324 conducted by Hazleton Laboratories America, Inc., Madison, WI, for Abbott Laboratories, North Chicago, IL; dated August 29, 1988.) Accession/MRID No. 408732-02.

5. REVIEWED BY:

Linda J. Plankenhorn, B.A.
Principal Reviewer
Dynamac Corporation

Signature: Linda J. Plankenhorn
Date: 10/2/89

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: 10/2/89

6. APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: Roman J. Pienta
Date: Oct 2, 1989

Irving Mauer, Ph.D.
EPA Reviewer, Insecticide/
Rodenticide Support
Toxicology Branch I
Health Effects Division
(H-7509C)

Signature:
Date: 10/02/89

Karl P. Baetcke Ph D
Chief, Insecticide/
Rodenticide Support
Toxicology Branch I
Health Effects Division
(H-7509C)

Signature: Karl P. Baetcke
Date: 10/03/89

7. CONCLUSIONS:

CORE Classification: CORE Guideline.

Dermal LD_{50}: >2.0 g/kg for both male and female rabbits.

Toxicity Category: III.

8. SUMMARY:

Five male and five female young adult New Zealand White rabbits [Hra:(NZW)SPF/Hazleton Research Products, Inc.], weighing from 2346 to 2692 g, received single dermal applications of gibberellins A4A7 at a dose level of 2.0 g/kg of body weight. Approximately 24 hours prior to dosing, the fur was removed from the back of each animal by clipping so that the clipped area made up approximately 20% of the total body surface. The test material, moistened with 0.9% saline, was applied to the clipped back of each animal at a dose level of 2.0 g/kg of body weight. The application site was covered with a gauze patch, secured with paper tape, and overwrapped with Saran wrap and Elastoplast tape. After 24 hours, the wrappings and patches were removed and the backs washed with lukewarm tap water and wiped with disposable paper towels.

The initial dermal irritation reading was made 30 minutes after removal of the test material using the Draize method: Subsequent readings of dermal irritation were made on days 3, 7, 10, and 14. Rabbits were observed for mortality and clinical signs at 1, 2.5, and 4 hours after dosing and twice daily thereafter for 14 days. Body weights were measured just prior to test material application and on days 7 and 14. At study termination, all animals were euthanized and subjected to gross necropsy examination.

There were no deaths during the study. Slight to moderate erythema was observed in 7 of 10 rabbits at the initial dermal-irritation reading (24 hours postdosing). Slight erythema was evident in 2 of 10 rabbits on day 3. By day 7, no signs of dermal irritation were evident in any of the test animals. At necropsy, multiple red areas at the treated skin site were noted for three male and two female rabbits; the study author concluded that the estimated dermal LD_{50} value of the test material was greater than 2.0 g/kg.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

The conduct and reporting of the study were adequate. A quality assurance statement was signed and dated August 30, 1988. The acute dermal LD_{50} value of the test material was reported to be greater than 2.0 g/kg. The test material belongs in Toxicity Category III.

10. CBI APPENDIX: Appendix A, Experimental Design, CBI pp. 23-28.

APPENDIX A

Experimental Design
(CBI pp. 23-28)

Gibberellins toxicology review

Page _____ is not included in this copy.

Pages 18 through 22 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

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X FIFRA registration data

___ The document is a duplicate of page(s) _____

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Attachment 1

Scale for Scoring Skin Reactions

Erythema

0 - None
1.0 - Slight
2.0 - Moderate (well defined)
3.0 - Severe (beet red)

Edema

0 - None
1.0 - Slight (barely perceptible to well defined by definite raising)
2.0 - Moderate (raised approximately 1 mm)
3.0 - Severe (raised more than 1 mm)

Atonia

0 - None
1.0 - Slight (slight impairment of elasticity)
2.0 - Moderate (slow return to normal)
3.0 - Marked (no elasticity)

Desquamation

0 - None
1.0 - Slight (slight scaling)
2.0 - Moderate (scales and flakes)
3.0 - Marked (pronounced flaking with denuded areas)

Coriaceousness

0 - None
1.0 - Slight (decrease in pliability)
2.0 - Moderate (leathery texture)
3.0 - Marked (tough and brittle)

Fissuring

0 - None
1.0 - Slight (definite cracks in epidermis)
2.0 - Moderate (cracks in dermis)
3.0 - Marked (cracks with bleeding)

EPA: 68D80056
DYNAMAC No.: 211-F
TASK No.: 2-11F
December 5, 1989

DATA EVALUATION RECORD

GIBBERELLINS

Acute Inhalation Toxicity Study in the Rat

STUDY IDENTIFICATION: Terrill, J. B. Acute inhalation toxicity study with gibberellins A4A7 (GA4A7) in the rat. (Unpublished study No. HLA 375-141 conducted by Hazleton Laboratories America, Inc., Rockville, MD, for Abbott Laboratories, North Chicago, IL; dated October 18, 1988.) Accession/MRID No. 408732-03.

NB [See additional comments by EPA reviewer, following p. 4 of this DER.] (*)

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 12/4/89

1. CHEMICAL: Gibberellins A4A7 (GA4A7); gibberellic acid (GA3); 2, 4a, 7-trihydroxy-1-methyl-8-methylenegibb-3-ene-1, 10-di-carboxylic acid 1,4-lactone.

2. TEST MATERIAL: Gibberellins A4A7 (GA4A7), Code 33691, lot No. 16-213-CD, contained approximately 90% active ingredient (48% gibberellin A4 and 42% gibberellin A7 by weight) and was described as a white powder.

3. STUDY/ACTION TYPE: Acute inhalation toxicity study in rats.

4. STUDY IDENTIFICATION: Terrill, J. B. Acute inhalation toxicity study with gibberellins A4A7 (GA4A7) in the rat. (Unpublished study No. HLA 375-141 conducted by Hazleton Laboratories America, Inc., Rockville, MD, for Abbott Laboratories, North Chicago, IL; dated October 18, 1988.) Accession/MRID No. 408732-03.

5. REVIEWED BY:

Linda J. Plankenhorn, B.A.
Principal Reviewer
Dynamac Corporation

Signature: Margaret E. Brower for
Date: December 5, 1989

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: December 5, 1989

6. APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: Roman J. Pienta
Date: December 5, 1989

Irving Mauer, Ph.D.
EPA Reviewer, Insecticide and Rodenticide Support
Toxicology Branch I
Health Effects Division
(H-7509C)

Signature:
Date: January 03 1990

Karl P. Baetcke, Ph.D.
Chief, Insecticide and Rodenticide Support
Toxicology Branch I
Health Effects Division
(H-7509C)

Signature:
Date: 1/13/90 but see Comments

25

7. CONCLUSIONS:

CORE Classification: CORE Minimum. No mortality was observed at the maximum attainable exposure level. The mean particle size was 5.83 microns with a geometric standard deviation of 1.67. The study author did not address whether particles of this size would be considered inhalable and inspirable for the test animal. Estimates of the percentage of particles that would be of inhalable size for the rat were not given.

Inhalation LC_{50} -- 4 hour exposure: >2.98 mg/L.

Toxicity Category: III.

8. SUMMARY:

A. Materials and Methods: Five male and five female Sprague-Dawley (Crl:CD BR) rats were exposed for 4 hours to a 5.0 mg/L target concentration of gibberellins A4A7 (GA4A7) as a respirable dust. Rats were observed for toxic effects at 30 minutes after exposure, 60 minutes after exposure, and once daily for 14 days thereafter. Body weights were measured prior to exposure on day 1, on day 8, and at terminal sacrifice on day 15. At study termination, all animals were euthanized and subjected to gross necropsy examination. The rats were 9-11 weeks old at study initiation and weighed between 224 and 306 g.

Rats were exposed by whole-body exposure to GA4A7 in a 100-L plexiglass exposure chamber operated in a dynamic mode. The test atmosphere was generated using a particle generator. House air at 30 psi was directed through a calibrated flowmeter and to the generator. The airflow rate to the generator was 56 liters per minute throughout the exposure. The resulting dust-laden atmosphere was directed undiluted from the particle generator to the exposure chamber. The concentration of test material in the chamber was determined gravimetrically by collection of the dust on filters. Concentration samples were collected from the middle of the chamber after 30 minutes of exposure and at approximately hourly intervals thereafter. Additionally, one gravimetric sample was taken from each end of the chamber in order to determine homogeneity of dust concentration. Airflow rate and chamber temperature and humidity were monitored continuously during exposure with specific readings recorded initially and at 30-minute intervals throughout exposure. Particle size distribution of the test material aerosol was determined twice during exposure with a cascade impactor; the mass median aerodynamic diameter (MMAD) and geometric standard

deviation (GSD) were calculated. Detailed materials and methods are given in Appendix A.

B. Results: The time weighted average (TWA) gravimetric (actual) and nominal exposure levels were 2.98 ± 0.313 and 88.5 mg/L GA4A7, respectively. The actual exposure level was considered the maximum attainable exposure level. The difference between actual and nominal exposure levels was attributed to sedimentation and/or impaction of dust in the exposure chamber. Particle size distribution measurements yielded an average MMAD of 5.83 microns and a GSD of 1.67 microns.

There were no deaths during the study. Clinical signs related to treatment were seen on the day of exposure and included compound on fur, salivation, crust on the eyes or nose, squinted eyes, lacrimation, rhinorrhea, and urine stains. Alopecia and crust on the eyes and nose were noted sporadically during the 2-week observation period; however, all animals were normal by day 15. No treatment-related effects on body weight were noted. No gross lesions were evident at necropsy. The median lethal concentration (LC_{50}) for a single 4-hour exposure is greater than 2.98 mg/L.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

We assess that the study was conducted in accordance with the regulatory guidelines. The reporting of the study was, in general, adequate. Gibberellins A4A7 (GA4A7) was tested at the maximum attainable exposure level which reached only 2.98 mg/L. Particle size distribution measurements yielded a mean particle size of 5.83 microns with a geometric standard deviation of 1.67 microns. The study author failed to address whether particles in this size range would be considered inhalable or inspirable for the test system. Estimates of the percentage of particles that would be of inhalable size for the rat were not given. Therefore, it is not possible to conclude that a sufficient percentage of the test material particles reached the alveoli of the lungs of the test animal.

A signed quality assurance statement, dated October 18, 1988, was included.

10. CBI APPENDIX: Appendix A, Methods, CBI pp. 12-16.

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(i)

(*)

[Additional Comments by EPA Reviewer

Although the Dynamac reviewer classified this study as "Minimum," a Core Grade is not being assigned to this acute inhalation assay because of substantive problems in achieving essential criteria of assay acceptance according to the Agency's test guidelines, when using the test material as applied (a white powder, 90% ai), difficulties acknowledged by all concerned in the appraisal of the study (see below). Since no deaths occured at the highest dose reported as attainable (2.98 mg/L), however, we can agree that the Toxicity Category is no worse than III.

1. The Dynamac review pointed out the failure of the study investigator/reporter (J.B. Terrill, Hazleton) to: a) Address the uncertainty that a particle size (MMAD) of 5.8 microns could be inspirable and/or inhalable by the rat respiratory system; or b) provide estimates of the requisite percentage of particles that would be of inhalable size for this test species.

2. One Agency inhalation expert considered the study unacceptable because: a) Particle size was "too large to be either inhalable or inspirable," and the study author failed to justify or explain why they could not achieve a one-micron size; b) the exposure chamber was inadequately described ("not standard"); and c) no justification or rationale was given for the calculated gravimetric (actual) concentration of 2.98 mg/L ("the maximum attainable concentration," according to the study author).

3. The study investigator subsequently submitted additional information in defense of his position on the maximally achievable test substance concentration and particle size, consisting of: a) A published article describing the design, construction and operation of a dynamic inhalation exposure chamber and controls; and b) an agenda and reports from a recent (August 10, 1989) meeting of other (outside) inhalation experts , addressing two critical issues, namely, the Agency's requirements that a limit test concentration be 5 mg/L, and the 1-micron particle size, as mandated by recent (inhouse) interpretations of FIFRA Test Guidelines for this type of assay.

4. Finally, the most recent FIFRA Test Guidelines for Acute Inhalation (OPP, November, 1984) state only that particle size for test compounds (TGAI, MP, or EP) be inhalable for man, i.e., aerodynamic diameters of 15 micrometers or less; and that a limit test can be first performed, at "an exposure of 5 mg/L (actual concentration of respirable substance) for 4 hours," or, "where this is not possible due to physical or chemical properties of the test substance, [at] the maximum attainable concentration . . . ."]

Attachments (DERs)

APPENDIX A

Methods
(CBI pp. 12-16)

Gibberellins toxicology review

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FUNDAMENTAL AND APPLIED TOXICOLOGY 2:33-37 (1982)

Design, Construction and Operation of a Simple Inhalation Exposure System

CRAIG S. BARROW and WILLIAM H. STEINHAGEN
Chemical Industry Institute of Toxicology, P.O. Box 12137, Research Triangle Park, North Carolina 27709

ABSTRACT

Design, Construction and Operation of a Simple Inhalation Exposure System. Barrow, Craig S. and Steinhagen, William H. (1982). *Fundam. Appl. Toxicol.* 2:33-37. An inhalation exposure system was designed from an all glass aquarium with a volume of 391 liters. A top for the chamber was fabricated from acrylic plastic and Teflon® (surface in contact with the test atmosphere) with an inlet, outlet, 5 sampling ports, and handles. Supply air to the chamber was charcoal and HEPA filtered. Air flow through the chamber is horizontal and can range from 50 to 200 liters/minute. Air flow is measured by a glass tube rotameter and regulated by polyvinyl chloride valves. Chamber air was HEPA and charcoal filtered prior to exhausting it to ambient air. The operating characteristics of this exposure system were assessed using test atmospheres of formaldehyde, chlorine, *n*-hexane and an aerosol of propylene glycol. Expressed as percent of a central sampling point the chamber concentrations of formaldehyde, chlorine and *n*-hexane ranged from 91.6% to 103.3% with an average of 97.9%. No differences in chamber distribution were noted at 78 or 130 liters/minute (corresponding to 12 and 20 chamber volumes/hour). For propylene glycol, the mass median aerodynamic diameter (MMAD) at a chamber air flow of 79 L/minute varied from 1.37 to 1.63 μm (σg range 1.34 to 1.43). Similar results were found at 130 liters/minute. During mass sampling a concentration gradient was found from the inlet to the outlet of the chamber and ranged from 55 to 99% ($\bar{x}$ = 78.5%) of the inlet concentration. This inhalation exposure system is very suitable for acute or sub-acute inhalation studies of gases or vapors but is only satisfactory for aerosols under certain operating conditions. It should prove to be particularly useful for laboratories wishing to set up a modest inhalation toxicology facility.

INTRODUCTION

The need to expose animals by inhalation to various airborne materials exists in many toxicology laboratories. In large toxicology facilities this is not a problem if there is an inhalation toxicology staff with exposure chamber facilities. However, much of the toxicological research today is conducted in smaller labs with little expertise in the area of chamber technology or fundamental aspects of generation and analysis of test atmospheres. As a result, research with airborne test agents in these labs is greatly hampered. Furthermore, the existing state-of-the-art technology utilized in large inhalation toxicology departments is usually not warranted in smaller labs for reasons of cost and space.

The sophistication of inhalation exposure systems has rapidly grown in the last several decades to include new chamber designs, new approaches to generating test atmospheres, and computer augmented control of exposure chambers and parameter measurement. However, there have been only a few published reports of simple inhalation exposure systems which may be readily adapted to any laboratory (Leach, 1963; Laskin and Drew, 1970; Montgomery, et al, 1976). The "Leach chamber" continues to be a useful apparatus but will not accommodate many animals (Leach, 1963). An exposure system designed from acrylic plastic (Laskin and Drew, 1970) is aerodynamically sound but may be incompatible with certain test atmospheres (i.e. organic solvents). Similarly, the chamber described by Montgomery is also constructed of plastic and can house only 6 small laboratory animals (Montgomery, et al, 1976). This exposure system is also overdesigned for the purposes of most inhalation exposures.

This paper describes an exposure system designed from a common all glass aquarium which was first used at the University of Pittsburgh nearly 10 years ago for exposure of mice to gases or vapors. The purpose of this report is to describe the design, construction and operating characteristics of this simple system.

METHODS

Exposure System

The exposure chamber is constructed from a 391 liter all glass aquarium[1] (Figure 1) which is readily available from pet supply stores. A top divided into two sections, each measuring 90.8 cm (35 in) x 43.2 cm (17 in), was fabricated from acrylic plastic and Teflon so that only Teflon was in contact with the test atmosphere. The Teflon which had a thickness of 3.2 mm (1/8 in) was secured to 6.4 mm (1/4 in) acrylic plastic with 6.4 mm (1/4 in) stainless steel machine screws. An inlet and outlet was provided for introduction and exhaust of the test atmosphere. These were fabricated from 25.4 mm (1 in) O.D. 316 stainless steel tubing and secured in the chamber top with bored-out 25.4 mm (1 in) Swagelok® stainless steel bulkhood unions.[2] Similarly, 5 sample ports were provided by using 6.4 mm (1/4 in) Swagelok stainless steel bulkhead adapters.[2] Thus, the only materials which came in contact with the test atmosphere were stainless steel, glass, and Teflon. The chamber top fit securely into a 8 mm (5/16 in) recessed ledge around the top of the aquarium. The top is easily sealed before each exposure by using strips of duct tape.

[1]Crystal Manufacturing Company, 1084 West 42nd St., Norfolk, Virginia 23508.
[2]Crawford Fitting Company, 29500 Solon Rd., Cleveland, Ohio 44139.

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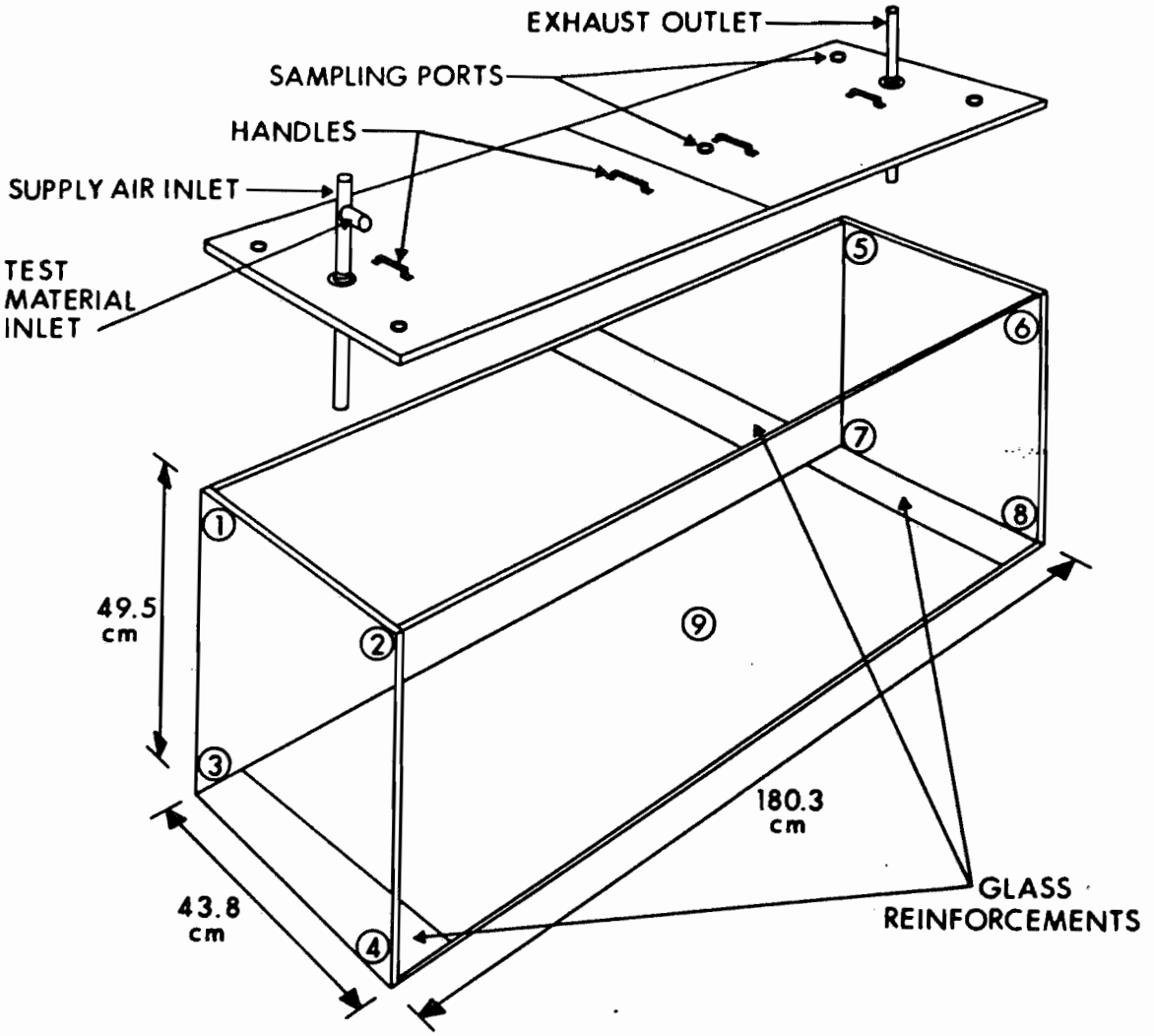

FIG. 1. Schematic of 391 liter exposure chamber showing dimensions and configuration of the chamber lid. Numbering (1-9) shows air sample locations for chamber distribution study. Sample points 1-8 correspond to the locations of the leading edges of the animal cages after placement in the chamber. Sample point 9 represents the mid-point equidistant from the top and bottom of the chamber.

The air handling system for the exposure chamber was made of 31.75 mm (1-1/4 in) schedule 40 polyvinyl chloride (PVC) pipe which was reduced with 19.1 mm (3/4 in) schedule 40 PVC pipe near the chamber inlet and outlet. Supply air, drawn from the room, was first filtered for particulate matter[3] followed by charcoal filtration. On the exhaust side of the chamber the contaminant was charcoal filtered prior to reaching the flowmeter.[4] Additional HEPA filtration may be added at this point for aerosol studies. Before exhausting to ambient air the chamber air was again charcoal and HEPA filtered.[5] A rotary vane vacuum pump[6] with a free air capacity of approximately 600 liters/min (21 ft^3/min) was used to exhaust the chamber (Figure 2). The pump had sufficient reserve capacity to ventilate the vaporization box containing the test chemical generation apparatus. For additional personnel protection, the vaporization box can be ventilated by a separate exhaust system in the event of equipment failure of the main pump. Figure 3 is a photograph of the exposure system in operation.

Operation characteristics

This exposure system was characterized for uniform chamber concentration with 4 separate agents. These included formaldehyde (HCHO), *n*-hexane (C_6H_{14}), chlorine (Cl_2), and a propylene glycol (PG) aerosol. All test atmospheres were evaluated at 78 and 130 liters/minute corresponding to 12 and 20 volume changes/hour, respectively. Under these conditions the chamber was operated at a sub-atmospheric pressure of approximately 2.5 to 5.0 cm of H_2O (1 to 2 in H_2O). Chamber concentration was checked at 9 separate points in the chamber (Figure 1). For the aerosol, samples were taken near the chamber inlet, exhaust and center.

Studies with HCHO, C_6H_{14}, and Cl_2 were conducted with the supply inlet located approximately 5 cm from the floor of the chamber and the exhaust equidistant from the top and bottom (Figure 2). For the PG aerosol the inlet was located approxi-

[3]Cambridge Filter Corporation, P.O. Box 1255, Syracuse, New York 13201.

[4]Brooks Instrument Division, Emerson Electric Company, 407 W. Vine Street, Hatfield, Pennsylvania 19440.

[5]Mine Safety Appliances Company, 600 Penn Center Boulevard, Pittsburgh, Pennsylvania 15235.

[6]Gast Manufacturing Corporation, P.O. Box 97, Benton Harbor, Michigan 49022.

mately 10 cm from the chamber top (Figure 2). All distribution studies were conducted with a full complement of 24 stainless steel cages in the chamber with no animals.

Test atmospheres of *n*-hexane, formaldehyde, or chlorine were evaluated by sampling sequentially beginning at sample point 1. For the aerosol, samples were taken randomly at the 3 locations.

Generation and analysis of test atmospheres

The HCHO gas was generated from paraformaldehyde,[7] a solid polymer of ~95% formaldehyde, by thermal depolymerization. This was accomplished by vaporizing paraformaldehyde which was placed in a stainless steel cannister and located inside a thermally-controlled oven. HCHO gas was carried by air (~500 mL/min) through a heated stainless steel tube and introduced at a right angle into the chamber air supply. HCHO chamber concentrations were monitored continuously by an infrared spectrophotometer[8] at a wavelength of 3.58 microns and pathlength of 20.25 meters. The infrared analyzer was calibrated with a paraformaldehyde permeation tube, whose permeation rate was quantitated by a colorimetric method based upon the reaction of HCHO with a chromotropic acid-sulfuric acid solution (Katz, 1977a).

Test atmospheres of *n*-hexane[9] were generated by metering the liquid at a constant rate[10] into a heated, 500 mL, three neck distilling flask. Dry, ultra zero air[11] was metered through the flask at a rate of 1.5 liters/min. The vaporized *n*-hexane was directed from the flask to the inlet of the exposure chamber where it was diluted. The concentration of *n*-hexane in the chamber was monitored continously by an infrared spectrophotometer at a wavelength of 3.416 microns and pathlength of 0.75 meters.

Test atmospheres of chlorine were obtained by metering a mixture of "chlorine-nitrogen" from a cylinder[11] with a calibratred flowmeter[12] into the supply inlet of the exposure chamber. Chlorine concentrations were measured by a colorimetric method based upon the oxidation of a methyl orange solution by free chlorine at a pH of approximately 3.0 (Katz, 1977b).

An aerosol of propylene glycol[13] was generated using a Dautrebande nebulizer operated at 0.5 to 0.7 kg/cm^2 (7 to 10 lbs/in^2) (Dautrebande, 1962). The flow rate through the impactor was approximately 45 L/min at 0.7 kg/cm^2. The particle

[7]Aldrich Chemical Company, 940 W. Saint Paul Avenue, Milwaukee, Wisconsin 53233.

[8]Foxboro Analytical, 140 Water Street, South Norwalk, Connecticut 06856.

[9]Phillips Petroleum Company, Bartlesville, Oklahoma 74004.

[10]Fluid Metering, Inc., P.O. Box 507, Oyster Bay, New York 11771.

[11]Matheson, P.O. Box 136, Morrow, Georgia 30260.

[12]Fischer and Porter Company, 295 Warminster Road, Warminster, Pennsylvania 18974.

[13]Fisher Scientific Company, Chemical Manufacturing Division, Fair Lawn, New Jersey 07410.

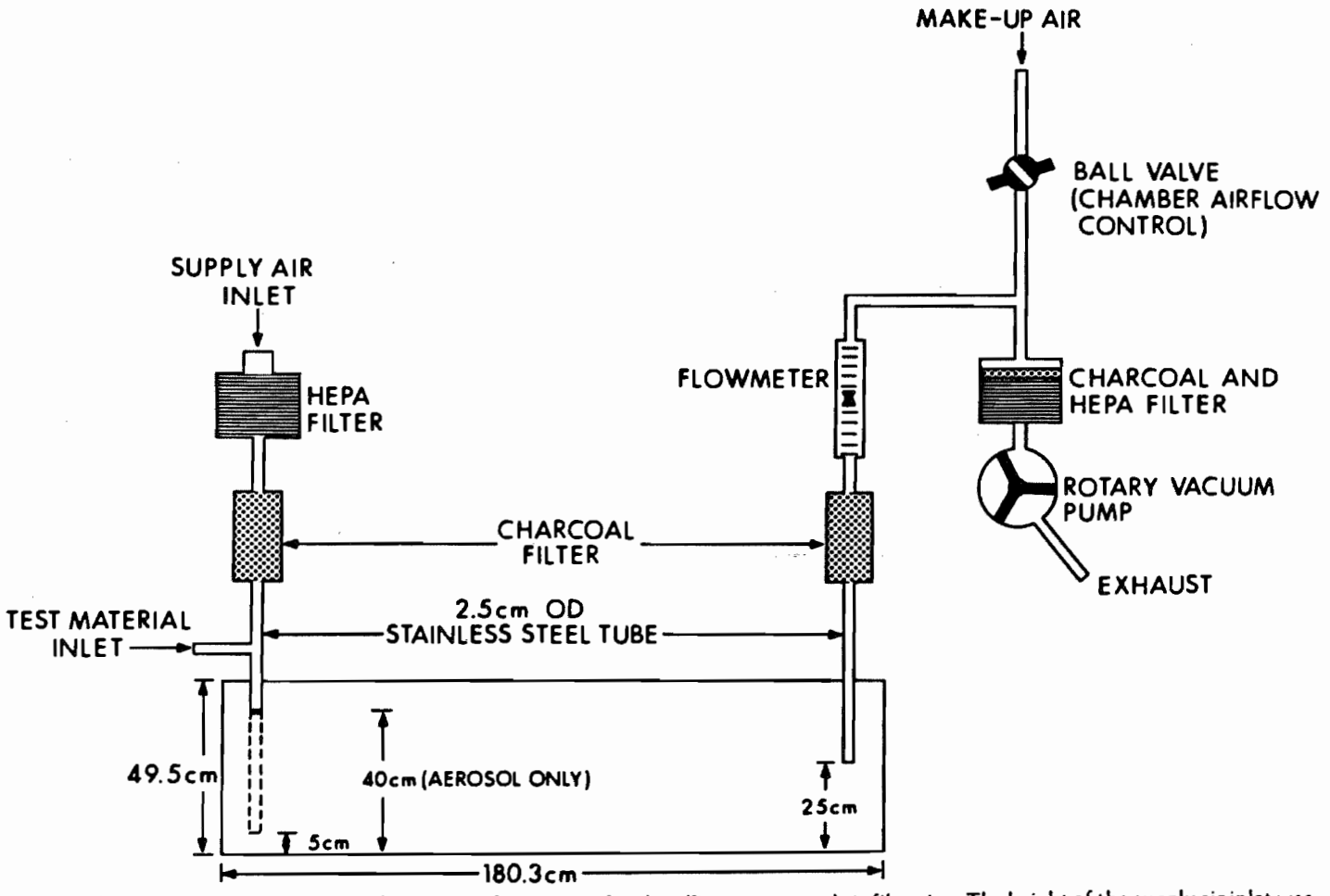

FIG. 2. Schematic of exposure system showing configuration of air handling system and air filtration. The height of the supply air inlet was adjusted depending upon whether gases or aerosols were used (see text).

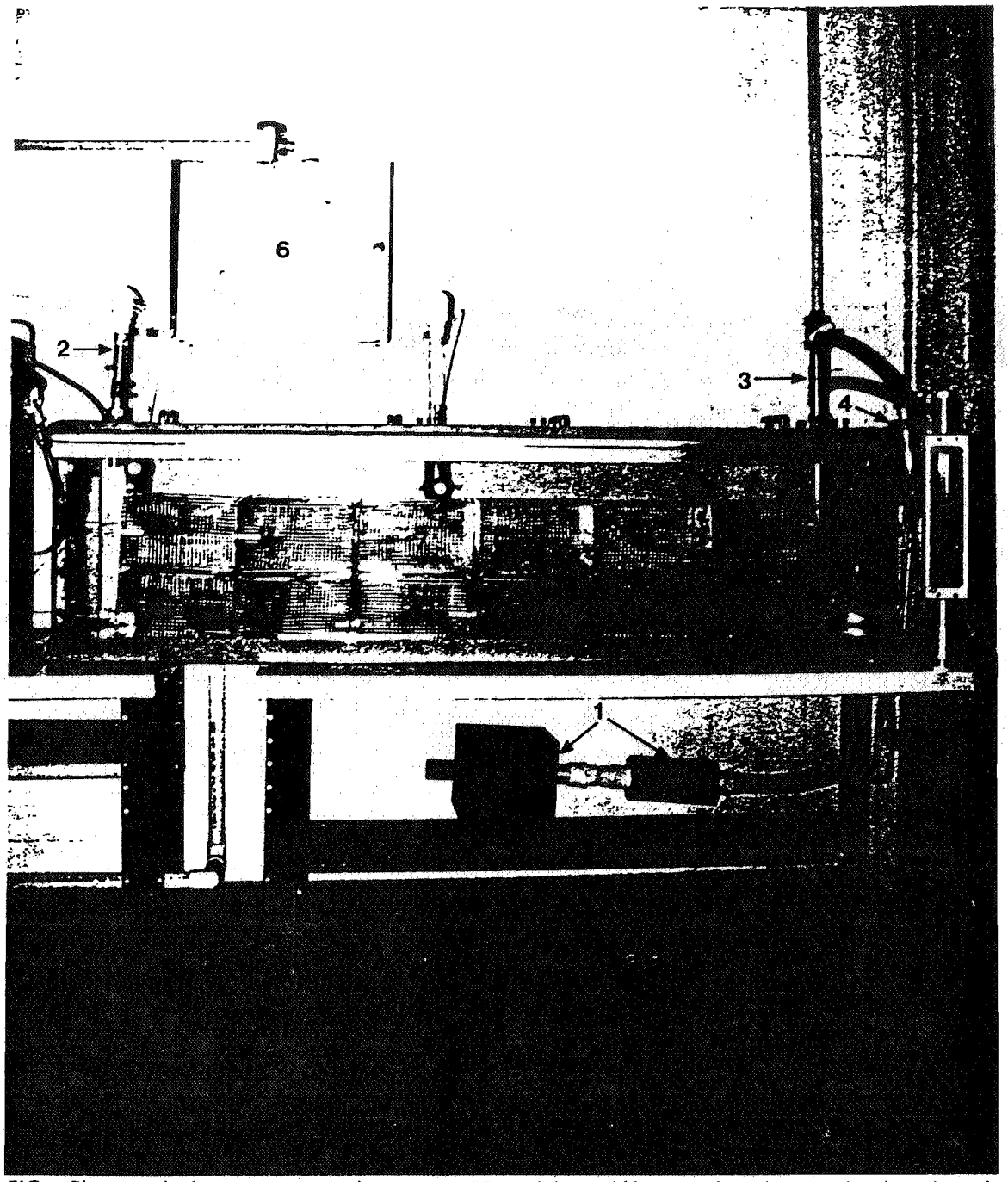

FIG. 3. Photograph of exposure system showing (1) HEPA and charcoal filtration of supply air (2) chamber inlet and (3) outlet (4) charcoal filtration of exhaust air (5) glass tube rotameter (6) vaporization box for containing generation apparatus and (7) 391 liter aquarium with stainless steel cages.

size of this aerosol was quantitated with a cascade impaction device.[14] Mass sampling for concentration was accomplished with a 25.4 mm (1 in) glass fiber filter[15] placed inside the chamber with sample collected at an air flow of approximately 4 liters/min. Impaction slides and glass fiber filters were weighed on an analytical balance[16] before and after sample collection.

[14]Delron Industries, Powell, Ohio (no longer in business).
[15]Whatman, Inc., 9 Bridewell Place, Clifton, New Jersey 07014.
[16]Mettler Instrument Corporation, P.O. Box 71, Hightstown, New Jersey 08520.

RESULTS

The concentration of HCHO, C_8H_{14}, or Cl_2 throughout the chamber ranged from 91.6% to 103.3% of the central sample point (location 9) with an average of 97.9% (Table 1). The chamber distribution was very similar at 78 and 130 L/minute for these 3 airborne chemicals. A slight concentration gradient was found from the chamber inlet to the exhaust with the concentration being approximately 4% less at the exhaust. This was found with each chemical at both 78 and 130 liters/minute

TABLE 1
Distribution of Chamber Concentration for Formaldehyde, n-Hexane, and Chlorine

Sample Location[A]	Air Flow = 78 L/min[B]	Air Flow = 130 L/min[B]
	Formaldehyde	
9 (mid-chamber)	20.7 ppm	12.2 ppm
1-8 ($\bar{x}$, range)	20.3 ppm (19.1 - 21.2)	12.0 ppm (11.4 - 12.3)
	n-Hexane	
9 (mid-chamber)	973 ppm	623 ppm
1-8 ($\bar{x}$, range)	966 ppm (950 - 990)	618 ppm (605 - 625)
	Chlorine	
9 (mid-chamber)	13.5 ppm	7.4 ppm
1-8 ($\bar{x}$, range)	13.0 ppm (12.4 - 13.5)	7.3 ppm (7.0 - 7.6)

[A]See Figure 1 for location
[B]Corresponds to 12 and 20 chamber volumes/hour, respectively.

Evaluation of the propylene glycol aerosol revealed a uniform particle size from chamber inlet to exhaust at 78 L/minute. This averaged 1.53 μm mass median aerodynamic diameter (SEM = $\pm$ 0.06 μm) with σg = 1.36 (SEM = $\pm$ 0.03). Similar results were obtained at 130 L/minute. An examination of the mass concentration (mg/liter) showed a rather large concentration gradient, irrespective of chamber air flow, which resulted in a lower concentration at the chamber exhaust. Expressed as percent of inlet concentration this was found to average 78.5% $\pm$ 8.7% (SEM, n = 6).

DISCUSSION

This simple inhalation exposure system has been successfully used in our laboratory for exposures of animals to chlorine, formaldehyde, ethylene, methyl chloride, and dimethylamine. The performance characteristics for gases and vapors reported here show that it is very suitable for these types of test atmospheres. It is particularly useful for highly reactive airborne agents which react with or adsorb onto chamber surfaces resulting in significant losses. Minimizing these losses enables much better control of inhalation exposures which is reflected in the ratio of actual to theoretical chamber concentration. During exposure of animals to Cl_2 this ratio normally approaches 80% in this exposure chamber compared to as low as 15% in conventional stainless steel chambers with similar animal loads (unpublished observation).

The stainless steel caging for the exposure system can singly house 24 adult rats, but we have exposed up to 48 rats in this chamber. Under the latter conditions, the animal load is less than 5.0%, well within the guidelines used for inhalation exposures. In addition, the wide variety of all glass aquariums now available makes it very easy to design smaller exposure systems. In our laboratory, we also maintain a series of 102 liter chambers and a 27 liter aquarium used exclusively for radiolabeled test atmospheres of gases or vapors. The latter exposure chamber fits conveniently in a standard size fume hood.

Because of aerodynamic considerations, the poorer performance of this exposure system with the propylene glycol aerosol is not surprising. However, these results were comparable to a recent report in which the concentration of a $CsCl_2$ aerosol (mass median aerodynamic diameter = 0.8 μm) was found to vary from 64 to 109% in a conventional 400 liter "Hinners type" chamber with vertical air flow (Drew, 1981). Similar results were obtained in a smaller "aquarium type" exposure chamber (102 liters) suggesting that this is not a problem associated with the larger chamber. Additional studies with other types of aerosols are necessary in order to determine the suitability of this type of chamber for aerosol studies. This exposure system may be adequate for aerosol exposures if the animal load is reduced, animals are kept in the same relative location from day to day, and the aerosol sample taken near them.

In conclusion, this exposure system is very satisfactory for any gaseous test atmosphere. Aerosols, however, should only be studied with appropriate precautions. Although it may be useful as an adjunct exposure system for existing inhalation laboratories, its greatest use should prove to be in laboratories wishing to set up a modest inhalation exposure facility. All parts are readily available and the chamber itself is easily replaced if broken. The total cost of all parts, excluding labor, should not exceed $2,000.

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Laskin, S. and Drew, R.T. (1970). An inexpensive portable inhalation chamber. *Am. Ind. Hyg. Assoc. J.* 31:645-646.

Leach, L.J. (1963). A laboratory test chamber for studying airborne materials. U.S. Atomic Energy Commission Progress Report UR 629. University of Rochester, Rochester, New York.

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EPA: 68D80056
DYNAMAC No.: 211-G
TASK No.: 2-11G
October 3, 1989

DATA EVALUATION RECORD

GIBBERELLINS

Primary Eye Irritation Study in Rabbits

STUDY IDENTIFICATION: Glaza, S. M. Primary eye irritation study of gibberellins A4A7 (GA4A7) in rabbits. (Unpublished report No. HLA 80602326 conducted by Hazleton Laboratories America, Inc., Madison, WI, for Abbott Laboratories, North Chicago, IL; dated August 29, 1988). Accession/MRID No. 408732-04.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Roman Prenta for

Date: Oct 3, 1989

42

1. CHEMICAL: Gibberellins A4A7 (GA4A7); gibberellic acid (GA3); 2, 4a, 7-trihydroxy-1-methyl-8-methylenegibb-3-ene-1, 10-dicarboxylic acid 1,4-lactone.

2. TEST MATERIAL: Gibberellins A4A7 (GA4A7), Code 33691, lot No. 16-213-CD, contained approximately 90% active ingredient (48% Gibberellin A4 and 42% Gibberellin A7 by weight) and was described as a white powder.

3. STUDY/ACTION TYPE: Primary eye irritation study in rabbits.

4. STUDY IDENTIFICATION: Glaza, S. M. Primary eye irritation study of gibberellins A4A7 (GA4A7) in rabbits. (Unpublished report No. HLA 80602326 conducted by Hazleton Laboratories America, Inc., Madison, WI, for Abbott Laboratories, North Chicago, IL; dated August 29, 1988). Accession/MRID No. 408732-04.

5. REVIEWED BY:

Linda J. Plankenhorn, B.A.
Principal Reviewer
Dynamac Corporation

Signature: Margaret E. Brower for
Date: 10/3/89

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: 10/3/89

6. APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: Roman J. Pienta
Date: 10-3-89

Irving Mauer, Ph.D.
EPA Reviewer, Insecticide and Rodenticide Support
Toxicology Branch I
Health Effects Division
(H-7509C)

Signature:
Date: 10/04/89

Karl P. Baetcke, Ph.D.
Chief, Insecticide and Rodenticide Support
Toxicology Branch I
Health Effects Division
(H-7509C)

Signature: KPB per
Date: 10/6/89

7. CONCLUSIONS:

CORE Classification: CORE Guideline.

Primary Eye Irritation Rating: Moderate irritant.

Toxicity Category: II--Corneal opacity reversible within 7 days or irritation persisting for 7 days. Corneal opacity was observed in one rabbit only at the 24-hour interval. All ocular irritation cleared by day 7.

8. METHODS AND RESULTS:

Three male and three female young adult New Zealand White rabbits (ages not specified; Hra:(NZW)SPF/Hazleton Research Products, Inc., Denver, PA), weighing from 2236 to 2376 g were randomly selected for this study. Pretest eye examinations were performed within 24 hours prior to test material administration using fluorescein dye procedures. A single dose of 0.06 g (0.1 mL weight equivalent) of the test material was placed in the conjunctival sac of the right eye. The eyelids of the treated eye were held together for 1 second to prevent loss of test material and then released. The contralateral eye served as the untreated control. Treated eyes were examined for ocular irritation at 1, 24, 48, 72, and 96 hours postdosing and on day 7 postdosing. At the 72-hour and 7-day examinations, sodium fluorescein was used to aid in revealing possible corneal injury. Ocular irritation was graded according to the Draize technique (see Appendix A). Individual data for each rabbit were presented.

Pain response (excessive pawing of the treated eye) was observed in all animals following instillation. Blanching of the conjunctivae was seen in six animals at 1 and 24 hours, in four animals at 48 hours, and in one animal at 72 and 96 hours. Petite hemorrhaging of the conjunctivae was observed in two animals at 1 hour and in one animal at 24 hours. Grade 1 iridal involvement was observed in all rabbits at the 1-hour interval and in two rabbits at the 24-hour interval. Iridal involvement had resolved by 48 hours postdosing. At the 1-hour observation, conjunctival irritation, which included redness (grade 3 in all animals), chemosis (grade 2 in all animals), and discharge (grade 3 in one male and one female; grade 2 in one male and two females; and grade 1 in one male) was recorded. Discharge had disappeared in all animals by the 72-hour observation, although one female showed a reappearance of discharge at the 96-hour interval. Chemosis was completely reversed in all animals by the 7-day observation. Redness decreased in severity over the 96-hour observation period and was completely cleared in all animals by day 7. A slight

corneal opacity was observed in one female at the 24-hour observation only.

The study author concluded that gibberellins A4A7 produced iridal involvement and moderate to severe conjunctival irritation in all animals. Corneal opacity was seen in one animal at the 24-hour observation only. All ocular irritation cleared within 7 days of test material instillation.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

We assess that the study was conducted properly and that the study author interpreted the data correctly. A quality assurance statement was signed and dated August 30, 1988.

10. CBI APPENDIX:

Appendix A, Scale for Scoring Ocular Lesions, CBI pp. 31-32; Appendix B, Experimental Design, CBI pp. 26-29.

APPENDIX A

Scale for Scoring Ocular Lesions
(CBI pp. 31-32)

Attachment 1

Scale for Scoring Ocular Lesions
(Draize[1] Technique)

	Value
1. Cornea	
A. Opacity - degree of density (area most dense taken for reading)	
No opacity	0
Scattered or diffuse area, details of iris clearly visible	1*
Easily discernible translucent areas, details of iris slightly obscured	2*
Opalescent areas, no details of iris visible, size of pupil barely discernible	3*
Opaque, iris invisible	4*
B. Area of cornea involved	
One-fourth (or less), but not zero	1
Greater than one-fourth, but less than one-half	2
Greater than one-half, but less than three-fourths	3
Greater than three-fourths, up to whole area	4

Score A x B x 5 Total maximum = 80

2. Iris	
A. Values	
Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)	1*
No reaction to light, hemorrhage, gross destruction (any or all of these)	2*

Score A x 5 Total maximum = 10

3. Conjunctivae	
A. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2*
Diffuse beefy red	3*

	Value
B. Chemosis	
No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2*
Swelling with lids about one-half closed	3*
Swelling with lids about one-half closed to completely closed	4*
C. Discharge	
No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

Score (A + B + C) x 2 Total maximum = 20

The total score for the eye is the sum of all scores obtained for the cornea, iris, and conjunctivae.

* Indicates positive effect. (FHSA Interpretation)

1. Draize, J. H. "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics - Dermal Toxicity", Association of Food and Drug Officials of the United States, pp. 46-59 (1975).

APPENDIX B

Experimental Design
(CBI pp. 26-29)

Gibberellins toxicology review

Page _____ is not included in this copy.

Pages 50 through 53 are not included in this copy.

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 product 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

X FIFRA registration data

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DYNAMAC No.: 211-H
TASK No.: 2-11H
October 3, 1989

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

DATA EVALUATION RECORD

GIBBERELLINS

Primary Dermal Irritation Study in Rabbits

STUDY IDENTIFICATION: Glaza, S. M. Primary dermal irritation study of gibberellins A4A7 (GA4A7) in rabbits. (Unpublished report No. HLA 80602325 prepared by Hazleton Laboratories America, Inc., Madison, WI for Abbott Laboratories, North Chicago, IL; dated August 29, 1988.) Accession/MRID No. 40877[illegible]-05.

32

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Roman J Prieto

Date: Oct 3, 1989

54

1. CHEMICAL: Gibberellins A4A7 (GA4A7); gibberellic acid (GA3); 2,4a, 7-trihydroxy-1-methyl-8-methylenegibb-3-ene-1, 10-dicarboxylic acid 1,4-lactone.

2. TEST MATERIAL: Gibberellins A4A7 (GA4A7), Code 33691, lot No. 16-213-CD contained approximately 90% active ingredient (48% gibberellin A4 and 42% gibberellin A7 by weight) and was described as a white powder.

3. STUDY/ACTION TYPE: Primary dermal irritation study in rabbits.

4. STUDY IDENTIFICATION: Glaza, S. M. Primary dermal irritation study of gibberellins A4A7 (GA4A7) in rabbits. (Unpublished report No. HLA 80602325 prepared by Hazleton Laboratories America, Inc., Madison, WI, for Abbott Laboratories, North Chicago, IL; dated August 29, 1988.) Accession/MRID No. 40877[illegible]-05.

32

5. REVIEWED BY:

Linda J. Plankenhorn, B.A.
Principal Reviewer
Dynamac Corporation

Signature: Margaret E. Brower for
Date: 10/3/89

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: 10/3/89

6. APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: Roman J. Pienta
Date: 10-3-89

Irving Mauer, Ph.D.
EPA Reviewer,
Insecticide and
Rodenticide Support
Toxicology Branch I
Health Effects Division
(H-7509C)

Signature:
Date: 10/04/89

Karl P. Baetcke, Ph.D.
Chief, Insecticide and
Rodenticide Support
Toxicology Branch I
Health Effects Division
(H-7509C)

Signature: K P B for I Mauer
Date: 10/06/89

7 55

7. CONCLUSIONS:

CORE Classification: CORE Guideline.

Primary Dermal Irritation Rating: Grade I--nonirritating.

Toxicity Category: IV--mild or slight irritation at 72 hours or no effects.

8. METHODS AND RESULTS:

Three male and three female young adult New Zealand White rabbits (ages not specified; Hra:(NZW) SPF/Hazleton Research Products, Inc., Denver, PA), weighing from 2398 to 2560 g were randomly selected for this study. Approximately 24 hours prior to dosing, the fur was clipped from the back and flanks of each animal. The test material (0.5 g of the powder) was applied to the intact skin of each rabbit and was moistened with 0.9% saline. The treated area was covered with a gauze patch (2.5 x 2.5 cm) secured with paper tape. The patch was occluded with Saran Wrap held in place by Elastoplast tape. Collars were used to restrain the test animals during the exposure period. After 4 hours, the wrappings and patches were removed. The test material was removed from the test site as thoroughly as possible without causing skin irritation using lukewarm tapwater and disposable paper towels. Thirty minutes following removal of the test material, the test site was scored for erythema and edema according to the Draize technique (see Appendix A). Subsequent dermal scores were recorded at 24, 48, and 72 hours after patch removal.

The test material produced no dermal irritation when applied to the skin of albino rabbits; thus, the author concluded that the test material was nonirritating.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

We assess that the study was conducted properly and that the study author interpreted the data correctly. A quality assurance statement was signed and dated August 30, 1988.

10. CBI APPENDIX:

Appendix A, Primary Skin Irritation Scoring Scale, CBI p. 23; Appendix B, Experimental Design, CBI pp. 18-21.

B

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APPENDIX A

Primary Skin Irritation Scoring Scale
(CBI p. 23)

Attachment 1

Primary Skin Irritation Scoring Scale

	Value
1. Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Highest possible erythema score	4
2. Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Highest possible edema score	4

APPENDIX B

Experimental Design
(CBI pp. 18-21)

Gibberellins toxicology review

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EPA: 68D80056
DYNAMAC No.: 211-I
TASK No.: 2-11I
October 18, 1989

DATA EVALUATION RECORD

GIBBERELLINS

Dermal Sensitization Study in Guinea Pigs

(Maximization Test)

STUDY IDENTIFICATION: Glaza, S. M. Dermal sensitization study of gibberellins A4A7 (GA4A7) in guinea pigs (maximization test). (Unpublished report No. HLA 80602327 prepared by Hazleton Laboratories America, Inc., Madison, WI, for Abbott Laboratories, North Chicago, IL; dated August 29, 1988.) Accession/MRID No. 408732-06.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir for

Date: Sept. 15, 1989

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1. CHEMICAL: Gibberellins A4A7 (GA4A7); gibberellic acid (GA3); 2,4a, 7-trihydroxy-1-methyl-8-methylenegibb-3-ene-1, 10-dicarboxylic acid 1,4-lactone.

2. TEST MATERIAL: Gibberellins A4A7 (GA4A7), Code 33691, Lot No. 16-213-CD, contained approximately 90% active ingredient (48% gibberellin A4 and 42% gibberellin A7 by weight) and was described as a white powder.

3. STUDY/ACTION TYPE: Dermal sensitization study in guinea pigs.

4. STUDY IDENTIFICATION: Glaza, S. M. Dermal sensitization study of gibberellins A4A7 (GA4A7) in guinea pigs (maximization test). (Unpublished report No. HLA 80602327 prepared by Hazleton Laboratories America, Inc., Madison, WI, for Abbott Laboratories, North Chicago, IL; dated August 29, 1988.) Accession/MRID No. 408732-06.

5. REVIEWED BY:

Linda J. Plankenhorn, B.A.
Principal Reviewer
Dynamac Corporation
Signature: Linda J Plankenhorn
Date: October 18, 1989

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation
Signature: Margaret E Brower
Date: October 18, 1989

6. APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation
Signature: William F. McLellan
Date: October 18, 1989

Irving Mauer, Ph.D.
EPA Reviewer, Pesticide and Rodenticide Support
Toxicology Branch I
Health Effects Division (H-7509C)
Signature:
Date: Oct. 24/89

Karl P. Baetcke, Ph.D.
Chief, Insecticide and Rodenticide Support
Toxicology Branch I
Health Effects Division (H-7509C)
Signature: Karl Baetcke
Date: 10/30/89

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7. CONCLUSIONS:

CORE Classification: CORE Minimum.

Skin Sensitization Potential: One of the 20 test animals showed a very slight dermal reaction to the challenge application of the test article 24 hours after removal of the challenge patches. Gibberellins A4A7 was classified as a weak skin sensitizer.

8. SUMMARY:

A. Materials and Methods: Forty-four young adult male albino guinea pigs of the Dunkin Hartley strain (Hazleton Research Products, Inc., Denver, PA), weighing from 432 to 576 g, were randomly selected for this study. The guinea pigs were individually housed and had continuous access to food and water. The guinea pig maximization method of Magnusson and Kligman (1970) was used to evaluate the contact sensitization potential of the test material.

A preliminary irritation screen was conducted to determine the appropriate dose levels to use for topical and challenge application. Four animals were treated with the test article at concentrations of 1, 10, 15, and 25% w/w in petrolatum. Each animal received two different concentrations of the test article. The test concentrations were applied to 2.0 x 2.0-cm Whatman No. 3 filter papers, which were placed on the shaved backs of the animals. The filter papers, were held in place with Blenderm and Elastoplast tapes. The dressings were removed after 24 hours, and the test sites were evaluated for erythema and edema 24 and 48 hours after removal of the dressings. These concentrations of the test article did not cause any dermal irritation. Based on the results of the irritation screen, a 25% w/w suspension of the test article in petrolatum was selected for both the topical induction application and the challenge phase in the definitive study.

For the definitive study, the remaining animals were randomly divided into two groups consisting of a test group and control group of 20 animals each. The hair was removed from a 4.0 x 6.0-cm area along the midline over the shoulder region of each animal in the test group. Each animal in the test group received duplicate intradermal injections (one on either side of the midline) of each of the following:

- 0.05 mL of Freund's complete adjuvant (FCA) solution (1:1 ratio of FCA and sterile water).

- 0.05 mL of a 5% w/v solution of the test article in sterile water.
- 0.05 mL of a 5% w/v solution of the test article in FCA solution (1:1 ratio of FCA and sterile water).

Six days after intradermal induction, the test areas were closely shaved and a 10% w/w suspension of sodium lauryl sulfate (SLS) in petrolatum was massaged into the skin at the injection site. Topical induction occurred 24 hours after SLS pretreatment (1 week after intradermal induction). A 25% w/w suspension of the test article in petrolatum was applied to a 2.0 x 4.0-cm patch of filter paper and placed over the injection sites. The patch was covered with Blenderm tape and secured with Elastoplast tape. The dressings and patches were removed after a 48-hour period. Control animals were not treated during the intradermal induction and topical induction phases.

Topical challenge was conducted 2 weeks after topical induction. The hair was removed from a 5.0 x 5.0-cm area of the right flank of each test and control (previously untreated) animal by shaving. The test article was applied as a 25% w/w mixture in petrolatum to a 2.0 x 2.0-cm Whatman No. 3 filter paper. The patch was placed on the shaved right flank of each test and control animal and was secured with Blenderm tape. The test sites were occluded with an overwrap of Elastoplast tape. The dressings and patches were removed at the end of a 24-hour contact period, and the test sites were wiped with a wet paper towel. Approximately 21 hours later, the test sites were closely shaved. Twenty-four and 48 hours after patch removal, the test sites were examined for erythema and edema. The reactions were scored using the following four-point scale: 0 = no reaction; 1 = scattered mild redness; 2 = moderate and diffuse redness; and 3 = intense redness and swelling. The frequency of positive responses at 24 and 48 hours after challenge, rather than the intensity of the responses, was used to determine the dermal sensitization potential of the test article. Animals were observed for clinical signs daily throughout the study; body weights were recorded prior to study initiation, at weekly intervals throughout the study, and at study termination. Detailed materials and methods are given in Appendix A.

B. Results:

One test group animal was found dead on day 13 of the study; this animal exhibited brown-stained abdomen and/or ataxia during the 3 days prior to its death. All other animals survived until study termination. A very slight

dermal reaction (grade 1) to the challenge application of the test material was observed in one test animal 24 hours after removal of the challenge patches. None of the control animals exhibited a dermal reaction to the challenge exposure.

The following rating scale was used to categorize the dermal sensitization potential of the test article:

Maximization Ratings

Sensitization Rate (%)	Classification
0	Not a skin sensitizer
1-9	Weak sensitizer
10-39	Mild sensitizer
40-69	Moderate sensitizer
70-100	Strong sensitizer

The study author concluded that the test material is a weak skin sensitizer in guinea pigs when tested by the Magnusson and Kligman maximization assay.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

We assess that the study data were adequate to support the author's conclusion that the test material is a weak skin sensitizer in guinea pigs.

The study was designed, conducted, and reported in accordance with guideline procedures with the following exception. The guidelines recommend that an appropriate positive control substance be periodically tested by laboratories performing skin sensitization studies to verify the responsiveness of the test system. Details of the positive control study (substance tested, method used, and time conducted) are to be included when reporting each skin sensitization study. No information concerning a positive control study was included in the report of the present study.

A quality assurance statement was signed and dated August 30, 1988.

10. CBI APPENDIX:

Appendix A, Experimental Design, CBI pp. 31-36.

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APPENDIX A

Experimental Design
(CBI pp. 31-36)

Gibberellins toxicology review

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