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EVALUATION OF OCULAR IRRITANCY POTENTIAL: SOURCES OF INTRALABORATORY VARIABILITY AND EFFECT OF DOSAGE VOLUME

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INTRODUCTION

Current standard methods for assessing the ocular irritancy potential of chemicals have been the subject of criticism from a number of directions. The best documented criticism is a study (1) which demonstrated the high degree of intralaboratory and interlaboratory variability of results from such tests using a 'standardized' methodology. This test has also been criticized based on the statistical invalidity of adding semi-quantitative scores obtained from three separate ocular tissues. Moreover, it has been suggested that a dosage volume of 0.1 mL, the standard liquid volume used, is extremely high relative to the surface area of the rabbit eye. This fact, together with known physiologic differences between rabbit and human eyes as well as human experience with eye irritants, has led to the conclusion that the current rabbit eye test may be an overly sensitive indicator of human risk (2). The most recent criticism of this test has been on humane grounds.

PURPOSE

The present investigation has been designed to study the following:

1. The sources of intralaboratory variability (reader, rabbit or test group) using standard Haskell testing procedures.
2. The dose-effect relationship using dose volumes of 0.10 mL and 0.01 mL of test compounds.
3. The sensitivity and reproducibility of the test using reduced dose volume.

PROCEDURE

Seven compounds (Appendices A and B) previously tested using standard Haskell Laboratory procedures were chosen. These compounds were selected on the basis of being liquids near neutral pH with observed, graded irritancy potential. Two were rated previously as severe irritants, 2 as moderate and 3 as mild. Five mL of liquid was transferred to a labelled foil-covered test tube and given a letter code (A-H). Compound G was the same as compound B.

Thirteen rabbits[1] were used each week for 6 weeks (72 rabbits total). Ten microliters each of compounds A-H, excluding G, were applied directly to the corneal surface of the right eye of 1 of 7 male albi. rabbits. The left eye was untreated and served as the control. The rabbit was released and the eye was not washed or manipulated in any way. Compound G was instilled in a similar manner into the right eye of 6 rabbits, but a volume of 0.10 mL was used. Compound G served as the internal control, and eyes dosed with it provided the data base for assessing sources of variability. Eyes were then scored at 1 and 4 hours and at 1, 2, 3, 7, 14, and 21 days (Haskell SOP, [redacted] by 2 independent and experienced readers (J. E. Henry and L. S. Ford). Observations of the cornea, iris and conjunctiva were made with an ophthalmoscope. Fluor-i-strip® staining was used at each examination after the day of treatment. Scores for conjunctival, corneal and iritic changes were recorded separately. To characterize the sources of variability, each of the indices (total corneal score or total conjunctival score) was analyzed independently using analysis of variance (3). Because of the magnitude of the variability change over time, each of the time periods was analyzed independently.

RESULTS

Sources of Variability

The 36 rabbits dosed with compound G (0.1 mL) served as internal controls to elucidate the sources of variability in eye irritancy scoring. Total scores for conjunctiva and cornea only are used in these determinations, since this compound did not have any iritic effect. The analysis of variance data is given in Appendices C and D. The results demonstrate that rabbit to rabbit differences are the greatest source of variability at any given time. At no time are the test groups a source of variability, suggesting that test group results are consistent and therefore reproducible. The only significant reader variability was measured at 4 hours post dosing. This is apparent for both corneal and conjunctival scores. This difference might be attributed to an artifact of the protocol. The study director was not aware that 1 reader consistently read before the other. At 4 hours post treatment the effects, in most cases, are reaching a maximum and may be changing rapidly. Consequently, the difference observed is probably due to a time effect, not reader variability.

[1] Animal strain: New Zealand White Rabbits
Supplier: B & H Rabbitry, Rockville, MD

Dose-effect Relationship

Appendices E and F show the individual scores (corneal and conjunctival) for rabbits dosed with 0.01 and 0.10 mL of the same compound. For this purpose, the first rabbit dosed with 0.1 mL of [redacted] each week was used for comparison with the rabbits dosed each week with 0.01 mL of [redacted]. The comparison between dose groups was made using the Mann-Whitney test (3). Each rabbit was assigned the mean score for the 2 readers at each time. The endpoints used for comparison were maximum score and time to recovery for the corneal and conjunctival index. Both endpoints for each index were significantly different at the $p \leq 0.05$ level.

Low-volume Eye Test

The median scores for corneal and conjunctival effects are plotted against time in Appendices G and H. By inspection, it is apparent that there is a graded response to this series of chemicals. The irritancy ratings which appear for each curve are those assigned to the respective compound when each was studied previously at a dose of 0.10 mL. A more rigorous analysis of these effects can be made if the time to recovery and maximum score are chosen as endpoints for comparison. By calculating the rank correlation at each end point between these scores and the qualitative scores obtained from previous tests, a quantitative measure of correlation between the 2 dose volumes can be derived. The correlation coefficients between the 2 dose levels in the cornea for time to recovery and maximum scores are 0.80 and 0.78, respectively. The correlation coefficients are 0.78 and 0.80 in the conjunctiva. All of these correlations are significant at the $p \leq .05$ level. Finally, since a clear dose-effect relationship has been demonstrated, it is possible that in some cases, mildly irritating compounds which would be detectable at a dose of 0.10 mL may be missed at the low volume. However, all 3 mild compounds tested had measurable corneal and conjunctival effects.

SUMMARY

Reader bias and test group to test group differences do not contribute significantly to the variability in scores obtained using a semi-quantitiative scale for assessing ocular irritancy. Most of the observed differences can be attributed to rabbit variability. However, the eye test is used routinely at Haskell only for effect/no effect determination of ocular irritancy potential. Therefore, the number of rabbits used for an eye test study should not be increased from 2 to 6 or more in order to increase the sensitivity of the test.

When tested at a dosage volume of 0.01 or 0.10 mL, a dose-effect relationship was discernible for [redacted] using either time to recovery or maximal effect as endpoints.

When tested at 0.01 mL, the same graded response was obtained for compounds which have been tested previously at 0.10 mL of pure material. However, based on the dose-effect relationship demonstrated in this study and based on previous Haskell data, all of these compounds are consistently less damaging when applied at a volume of 0.01 mL. In light of these results, reducing the volume applied to the eye apparently moderates the responses observed with most compounds and may be a more realistic approach to assessing risk of eye irritation in humans. It is therefore recommended that a dose volume of 0.01 mL or its weight equivalent be used in routine testing [redacted] of chemicals for eye irritancy potential at Haskell Laboratory.

APPENDIX A - TEST COMPOUNDS

Material Tested	Haskell No.	Date Received	Other Codes and Names	Letter Code Used In Study	Material Submitted By
[redacted]	13,891	12/10/80	[redacted]	H	[redacted] Textile Fibers Dept.
[redacted]	13,893	12/12/80	[redacted]	C	[redacted] Chemicals & Pigments Dept. Jackson Lab
[redacted]	13,197	8/10/79	[redacted]	D	[redacted] Chemicals & Pigments Dept. Jackson Lab
[redacted]	13,895	12/12/80	[redacted]	E	[redacted] Chemicals & Pigments Dept. Jackson Lab
[redacted]	13,896	12/10/80	[redacted]	B,G	[redacted] Chemicals & Pigments Dept. Jackson Lab
[redacted]	13,897	12/05/80	[redacted]	A	[redacted] Central Research & Development Dept. Haskell Laboratory
[redacted]	13,611	6/23/80	[redacted]	F	[redacted] Chemicals & Pigments Dept. Jackson Lab

APPENDIX B

CHARACTERISTICS OF TEST COMPOUNDS

[redacted] Composition: [redacted]

Contaminants: [redacted]

[redacted] Composition: [redacted]

Purity: [redacted]

[redacted] Composition: [redacted]

Contaminants: [redacted]

[redacted] Purity: [redacted]

[redacted] Purity: [redacted]

Contaminants: [redacted]

[redacted] Purity: [redacted]

[redacted] Purity: [redacted]

Contaminants: [redacted]

APPENDIX C

ANALYSIS OF VARIANCE
CONTROL CORNEAL SCORES[a]

Time Post-Dosing	Variance				% Of Variance
	Reader	Group[b]	Rabbit	Residual	Due To Rabbits
1 Hr.	NS[c]	NS	0.91	1.10	45.1
4 Hr.	0.25	NS	0.25	0.67	21.4
1 Day	NS	NS	0.33	0.54	38.1
2 Day	NS	NS	0.30	0.86	25.8
3 Day	NS	NS	3.03	0.62	83.1
7 Day	NS	NS	1.71	0.93	65.1
14 Day	NS	NS	1.73	0.38	81.8
21 Day	NS	NS	1.75	0.05	97.2

a) each value is based upon the total corneal score obtained from 36 rabbits.
b) group to group differences were assessed on the basis of 6 rabbits/week for 6 weeks
c) Not significant

APPENDIX D

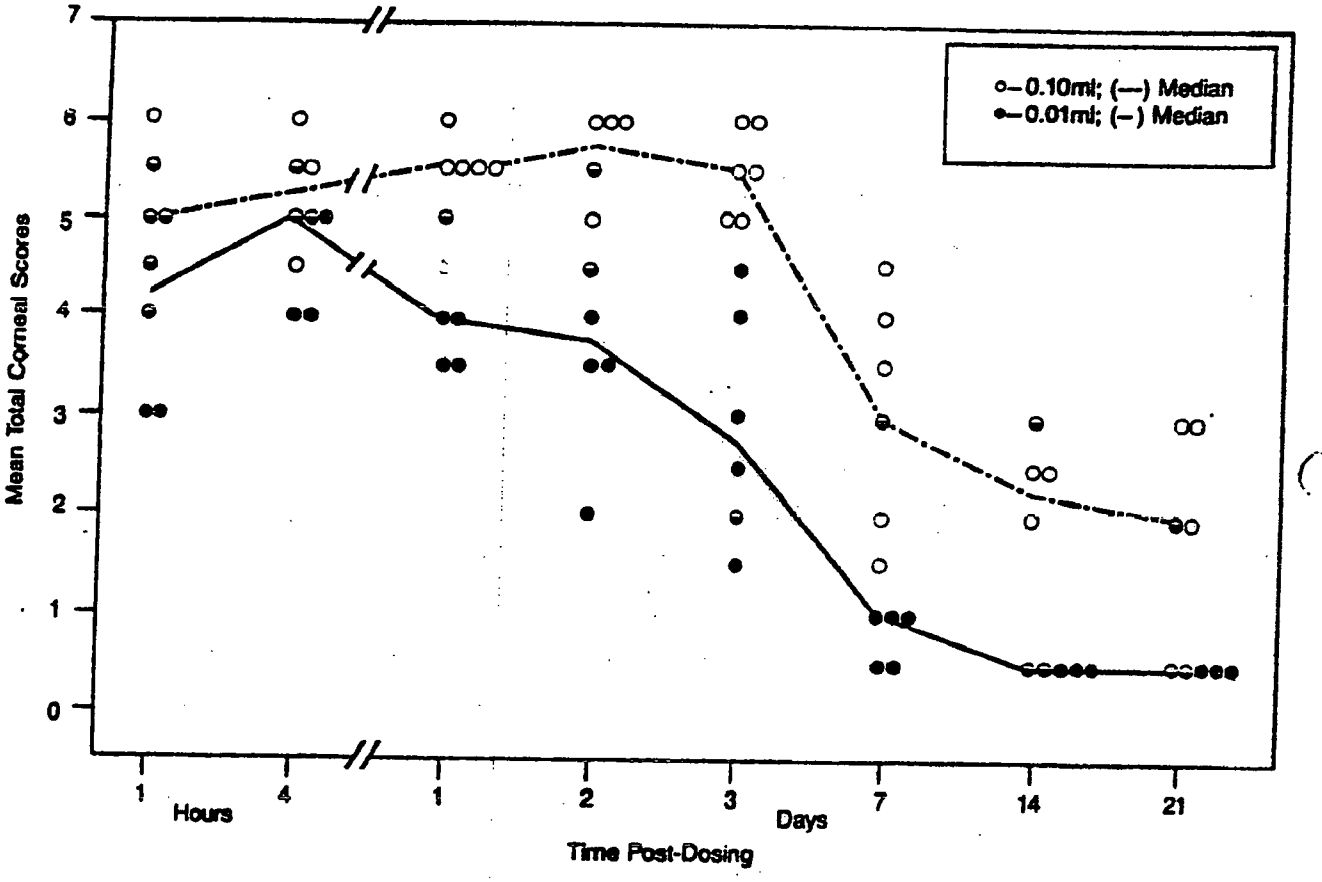
ANALYSIS OF VARIANCE
CONTROL CONJUNCTIVAL SCORES[a]

Time Post-Dosing	Variance				% Of Variance
	Reader	Group[b]	Rabbits	Residual	Due To Rabbits
1 Hr.	NS[c]	NS	1.60	0.95	61.1
4 Hr.	0.99	NS	0.75	0.53	33.0
1 Day	NS	NS	1.43	0.73	66.2
2 Day	NS	NS	3.00	0.62	82.9
3 Day	NS	NS	3.01	0.48	86.0
7 Day	NS	NS	1.45	0.39	78.8
14 Day	NS	NS	0.64	0.19	77.1
21 Day	NS	NS	0.04	0.05	30.8

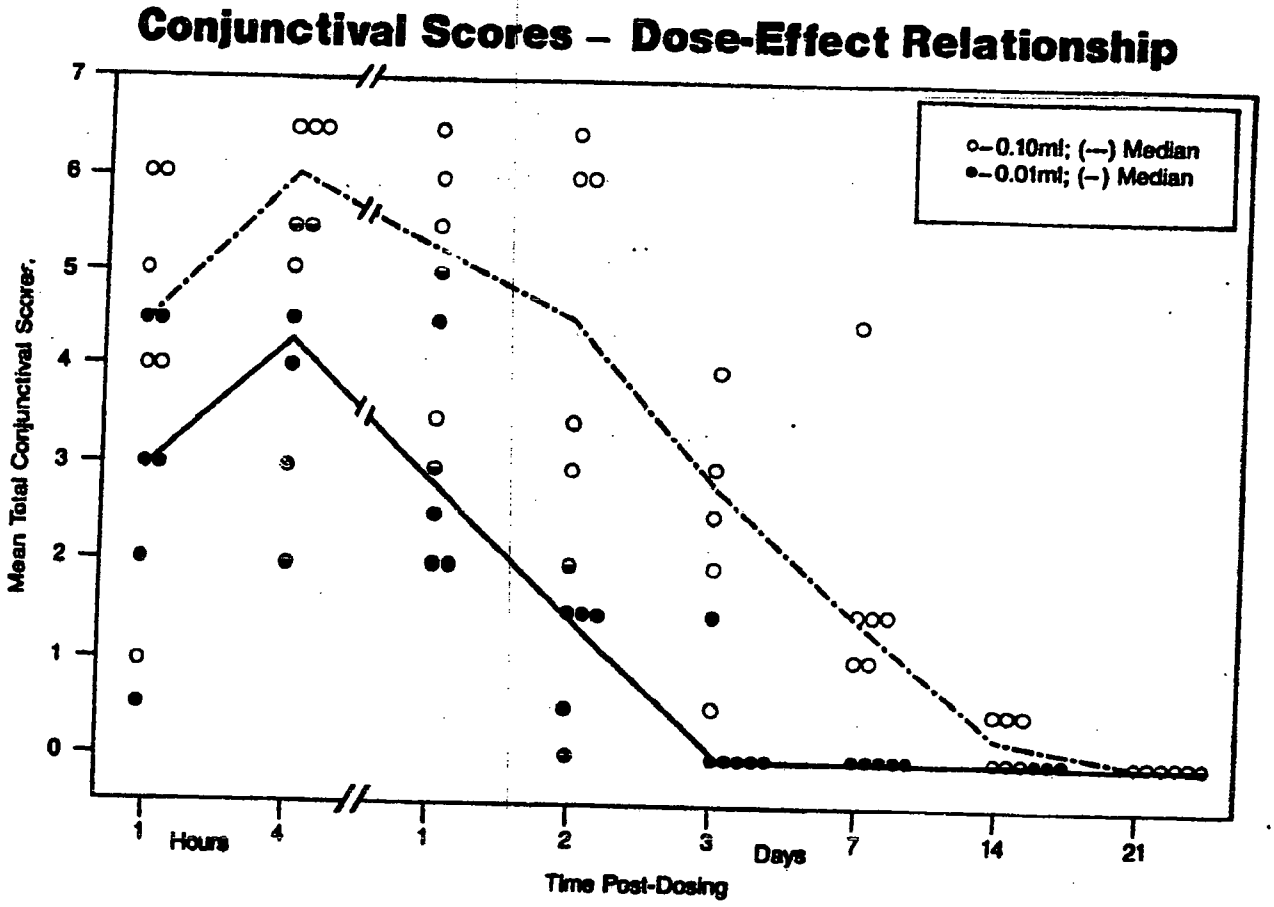
a) each value is based upon the total conjuctival score obtained from 36 rabbits
b) group to group differences were assessed on the basis of 6 rabbits/week for 6 weeks
c) not significant

APPENDIX E

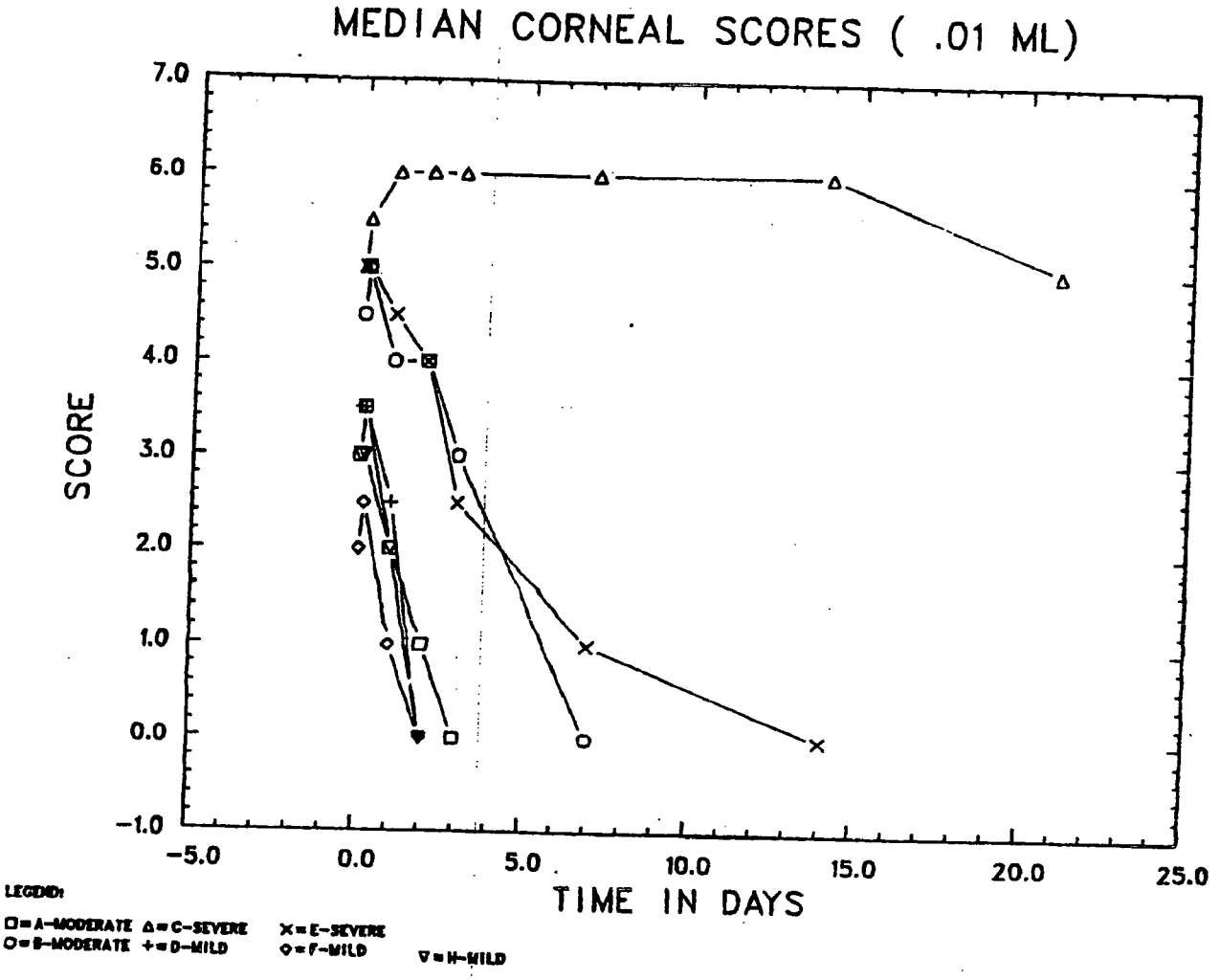
Corneal Scores – Dose-Effect Relationship



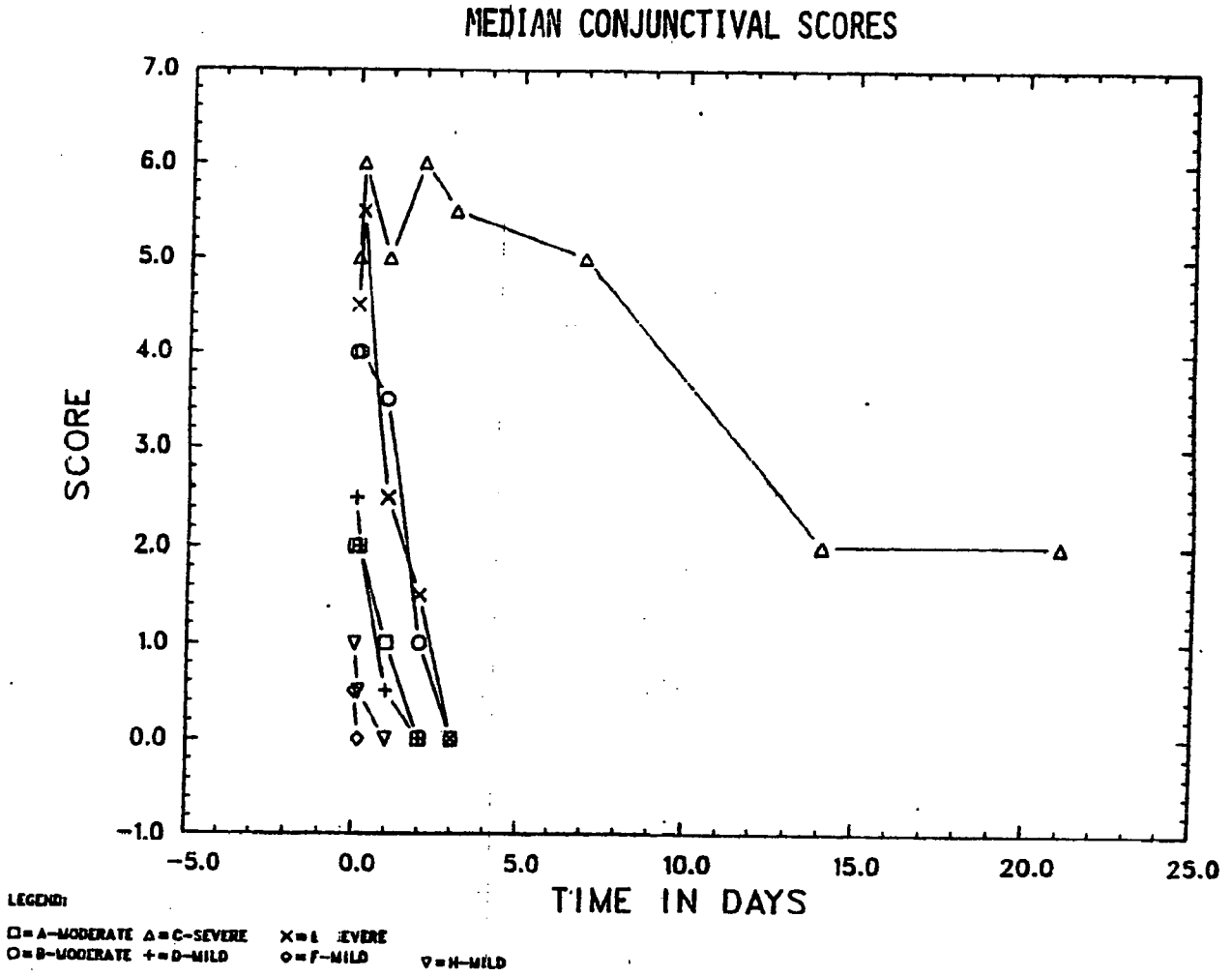
APPENDIX F



APPENDIX G



APPENDIX H



APPENDIX I

Literature Cited

(1) Weil, C. S. and Scala, R. A. (1971). "Study of Intra-and Inter-laboratory Variablility in the Results of Rabbit Eye and Skin Irritation Tests". Toxicol. Appl. Pharmacol., 19:276-360.

(2) Griffith, J. F., Nixon, G. A., Bruce, R. D, Reer, P. J., Bannan, E. A., (1980). "Dose-Response Studies with Chemical Irritants in the Albino-Rabbit Eye as a Basis for Selecting Optimum Testing Conditions for Predicting Hazard to the Human Eye". Toxicol. Appl. Pharmacol., 55:501-513.

(3) Snedecor, G. W. and Cochran, W. G., (1980) Statistical Methods, The Iowa State University Press, Ames, Iowa.