PB90-146309

EPA560/6-89-002

# Summary of the EPA Workshop on Carcinogenesis Bioassay via the Dermal Route
April 28-29, 1987
Washington, D.C.

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50272-101

**REPORT DOCUMENTATION PAGE**

1. REPORT NO. EPA 560/6-89-002
2.
3. Recipient's Accession No

4. Title and Subtitle
Summary of the EPA Workshop on Carcinogenesis Bioassay via the Dermal Route

5. Report Date
April 28-29, 1987
6.

7. Author(s)
Oncology Branch/HERD/OTS

8. Performing Organization Rept No

9. Performing Organization Name and Address
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10. Project/Task/Work Unit No
62
11. Contract(C) or Grant(G) No
(C) 68-01-7266
(G)

12. Sponsoring Organization Name and Address
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M. Street, S.W.
Washington, DC 20460

13. Type of Report & Period Covered
Final Technical Report
08/28-29/87
14

15. Supplementary Notes

16. Abstract (Limit 200 words)

Traditionally, the oral route has been the most common route of administration in bioassays which tested the potential carcinogenicity of chemicals. Regulatory agencies, however, prefer to have test chemicals applied by the same route as expected human exposure, whenever possible. Since human exposure to industrial chemicals is frequently via the dermal route, this has become a route of choice for animal testing of certain chemicals. However, protocol design for dermal bioassays presents many unique problems which must be addressed before guidelines for bioassays by the dermal route can be formulated. Furthermore, it may be feasible to develop a limited dermal protocol to screen certain classes of chemicals such as acrylates/methacrylates. Recognizing the need for this workshop, it was designed in two distinct parts; to address the problems inherent in the development of (1) a generic protocol for dermal bioassays and, (2) a specific limited dermal bioassay protocol for acrylates/methacrylates.

17. Document Analysis a. Descriptors
Dermal Bioassay; Acrylates/Methacrylates; carcinogenesis

b. Identifiers/Open-Ended Terms
Identifiers/open-ended terms
Dermal bioassay protocol; limited dermal protocol for screening acrylates/methacrylates; skin irritation; MTD

c. COSATI Field/Group

18. Availability Statement
Document is available to the public through the National Technical Information Service, Springfield, VA 22151; unlimited release

19. Security Class (This Report)

20. Security Class (This Page)

21. No. of Pages
9

22. Price

(See ANSI-Z39.18) See Instructions on Reverse

OPTIONAL FORM 272 (4-77)
(Formerly NTIS-35)
Department of Commerce

The need for this workshop arose from the fact that in bioassays to test the potential carcinogenicity of chemicals the oral route has been the most common route of administration used Regulatory agencies however prefer to have test chemicals applied by the same route as expected human exposure whenever possible Since human exposure to industrial chemicals is frequently via the dermal route, this has become a route of choice for animal testing of certain chemicals However protocol design for dermal bioassays presents many unique problems which must be addressed before guidelines for bioassays by the dermal route can be formulated Furthermore, it may be feasible to develop a limited dermal protocol to screen certain classes of chemicals where exposure is known to occur primarily via the dermal route such as the acrylates/methacrylates This workshop was designed in two distinct parts to address the problems inherent in the development of (1) a generic protocol for dermal bioassay and (2) a specific limited dermal bioassay protocol for acrylates/methacrylates

The objectives of the workshop were therefore to

(1) define and address key issues involved in designing a protocol for testing the carcinogenicity (both systemic and dermal) of substances by the dermal route

(2) use the results of these discussions to explore the feasibility of developing a limited dermal protocol for the screening of acrylates/methacrylates for oncogenic potential

Experts who participated in the workshop included John Clary Lionel DePass William Eastin James McDermott Michael Moore Stephen Nesnow and Andrew Sivak who were selected for their expertise in chemical carcinogenesis including special empnasis on dermal or acrylate carcinogenesis and on pharmacokinetics Gary Johnson and David Strayer, two pathologists specializing in the skin and Ralph Kodell and Joseph Haseman, two statisticians knowledgeable in experimental design The written comments of these experts in response to a questionnaire aimed at identifying and probing issues and problems related to the stated objectives formed the basis for the discussions at the 2 day workshop

The consensus of the group stated at the outset was that alternative experimentation to animal testing should always be considered and that unnecessary replication should be avoided

## Recommendations for the Design of a Protocol for a Carcinogenesis Bioassay via the Dermal Route

### Concerning Species and Strain Selection

- It is most desirable to have two species of animal for dermal bioassay
- One species should be the mouse however selection of the strain of mouse can only be made after review of the existing data on the relative susceptibility of different mouse strains to skin

irritation and to tumor induction following application of chemicals to the skin

- Both the rat and the hamster may be viable options for the second species, however, careful analysis of the existing data on the use to date, of these two species in carcinogenesis studies by the dermal route is necessary before a decision can be made

- The rabbit and guinea pig were ruled out as feasible species for dermal bioassay testing, mainly because of technical difficulties, expense and lack of a historic database

- If after review of the existing databases on the rat and the hamster a second species can not be identified that is acceptable for dermal bioassay testing, then the possibility of testing in the rat by the oral route in conjunction with the dermal testing of the mouse was suggested

- Another less desirable possibility suggested by the group was that review of the existing database on different strains of mice used in dermal testing may reveal that there is a difference between mouse strains with respect to susceptibility to irritation, and that this could possibly be the basis for the selection of two different strains of mouse to be used in dermal bioassay testing rather than two different species

Dose Selection

- Regardless of whether the toxicity endpoint for estimating the MTD involves the skin or is systemic, it was agreed that a 90-day dose finding study is necessary to determine an MTD for the long term bioassay. Short-term toxicity, absorption/pharmacokinetic data should be available prior to conducting the 90 day dose-finding study. Microscopic examination of the skin should be part of the 90 day study. Skin absorption studies also should be done at several time points during the study and at the end of the 90 days to provide data on differences in absorption with time during exposure

- It is imperative that the same test animal (species and strain) be used for the subchronic range-finding studies as for the long-term bioassay regardless of what species may have been used in an acute or subchronic irritation study for the same chemical

- The Draize skin irritation scoring code was deemed unacceptable for use in the range-finding study for a 2-year dermal bioassay since it relies only on visual evidence of irritation and was developed specifically for rabbits. The consensus was that histopathologic evidence of irritation must be considered when determining the MTD and other doses to be employed in the long-term bioassay, and that another panel of experts comprised of pathologists and toxicologists should be convened to devise a

scoring method for visual and microscopic irritation of the skin

- It was also agreed that when the skin response is used to determine the MTD, the desired endpoint is the highest dose where the integrity of the skin is not destroyed A more explicit definition is needed for deciding when the "integrity of the skin" is destroyed, and this must also be decided by the above-mentioned panel The consensus of the group was that hyperplasia and hyperkeratosis is acceptable, that ulceration of the skin is unacceptable, and that factors such as changes in the skin in regards to flux, permeability, etc , as well as the reversibility of any changes seen must be considered in estimating an MTD

- It was generally agreed that 3 dose levels (plus a control) be used and the MTD should be the highest dose in the long-term bioassay When skin irritation is a factor, the ideal choice for the other two doses would be one dose that is minimally irritating and one that is the highest non-irritating dose Another basis for choosing doses considers chemical-specific data (i e absorption, kinetics, toxicity, etc ) and therefore it is difficult to assign an arbitrary and generic method for selecting doses for a long-term study The suggestion was also made that one of the doses should approximate the occupational exposure level if possible

Specific Considerations when Applying Chemicals to the Skin

- It was agreed that use of the automatic pipette was an acceptable and the most accurate method for delivery of the desired dose to the skin Spreading the test material with the disposable pipette tip was recommended in those instances where the material is particularly viscous

- The consensus was that the volume of application should be 50 µl for the mouse (not to exceed 100 µl) and a limit of 300 µl for the rat

- The following points are to be considered in selecting a vehicle

  Solubility and suspendability
  - High volatility
  - Toxicity
  - Permeability
  - Does not induce tumor formation

  Vehicles discussed include acetone water, mineral oil, acetone + cyclohexane (1 1), ethanol + water, and toluene If occupational exposure to a chemical occurs in conjunction with a particular vehicle then that vehicle should be considered The vehicle used in the toxicity studies should be the same as that used in the absorption studies

- The consensus was that the interscapular region is the desired site of application. The area of application should not exceed 10% of the animal's total body surface area

- Duration of Study. the consensus was that a dermal bioassay should be a lifetime study, that is, two years for mice rats or hamsters

- Design of Study. the design of the study should attempt to approximate continuous exposure. The frequency of application should be determined prior to or concurrent with the 90-day study. Five applications/week is the preferred frequency of application but 3 applications/week would be acceptable

  The factors to be considered in determining dosing frequency include

  - Irritation
  - Effects of clipping
  - Toxicity
  - Absorption, distribution and excretion of the test substance

Special Concerns that Arise when Using the Dermal Route

1 Problems concerned with skin clipping/shaving

There was an agreement that nicking due to clipping should not be a problem if you have proper technical expertise and that irritation due to clipping is generally not a problem provided that a minimum of 24 hours is allowed for the skin to recover before the next dosing of the animal. It was agreed that the hair cycle is not an issue for dermal bioassays

2 How to prevent exposure by routes other than dermal

The consensus was that some exposure by other routes is a problem that "we all have to live with" if we are going to study the toxicity/carcinogenicity of chemicals by the dermal route of exposure. Practical ways of minimizing exposure by other routes are to house the animals individually (preferably in wire-mesh-bottom cages) and to apply the chemical to the upper back region

3 Is it appropriate to minimize irritation/local toxicity by alteration of the dosing schedule and site of application

If the MTD of the test substance has been overestimated, altering the concentration of the test material is preferable to changing the dosing schedule. No one favored altering the site of application

4 When the test chemical is a liquid or is applied in solution should the dosage be expressed per surface area of per body weight

The consensus was that per body weight is the preferred way to express dosage

5 When different dose levels of a chemical in solution are used, is it preferable to keep the concentration the same and alter the volume applied or to always apply the same volume and to adjust the concentration

The consensus was that the volume should be kept constant and the concentration of the test solution should be altered when different dose levels are used

6 Should the level of exposure to the test material be constant as the animal grows, and if so, how should this be done

Three options were proposed in answer to this question

a Keep a constant volume and alter the concentration of the dose solution as the body weight of the animal changes in order to maintain a constant weight/body weight dosage

b Keep a constant concentration of dose solution and alter the volume as the body weight of the animal changes to maintain a constant weight/body weight dosage

c Keep the dose solution volume and concentration constant throughout the duration of testing

The consensus was that option "c" is really the only practical option but if the MTD is not based on irritation as the endpoint then options "a" or "b" can be considered With option "c" dose can still be expressed as dose/body weight by using the total dose administered/average body weight throughout the study

There was also agreement on which questions could not be answered at this time and on what follow up information is necessary in order to arrive at these answers

1 A thorough literature search into the use of rats and hamsters in long-term carcinogenesis studies by the dermal route, and the results of these studies must be made Analysis of this database should then provide the information needed for establishing the usefulness of these animals as a second species for the dermal bioassay

2 A similar database on the relative susceptibility of different mouse strains to skin irritation and to tumor induction following application of chemicals to the skin is also needed Mice have been used extensively in skin tumorigenesis studies, so there should be a wealth of data which, on analysis, would indicate which mouse strains are the most appropriate for the purposes of dermal bioassay

3 It is imperative that we establish a skin irritation scoring code including histopathologic evidence, for the rodent species to be used in the long-term dermal bioassay This is especially needed in the dose-selection phase The panel of experts were in agreement that this scoring code should be arrived at in a second workshop composed primarily of pathologists, but also including toxicologists

4 The reasons for including toxicologists in this second workshop is that it is anticipated that once scoring levels of irritation are established this same group will also define that level of irritation to be accepted as the endpoint in determing the MTD when skin is the target organ for toxicity

Recommendations for the Design of a Limited Dermal Protocol for the Screening of Acrylates/Methacrylates

The consensus was that a limited dermal bioassay was an acceptable option for screening the carcinogenicity of acrylates/methacrylates It is to be used to obtain a yes/no answer on the carcinogenicity of this class or members of this class, of chemicals and not for quantitative risk assessment The acrylates/methacrylates have been shown to induce skin tumors and possibly lymphomas in mice, and therefore the need exists to look at both dermal and systemic carcinogenicity of these chemicals

- The consensus was to use one species, the mouse, for this limited protocol The strain of mouse will be selected after examining the database in regards to sensitivity to skin irritation and susceptibility to tumorigenesis of different mouse strains

- The duration of the study is not to be limited, that is it is to be at least an 18-month study

- It was agreed to use only one sex, that is, male mice in this limited protocol, since systemic tumors have been found only in males, and no sex difference has been noted in skin tumor susceptibility with acrylates

- A 90-day range-finding study is recommended The number of doses used depends on the chemical Preliminary acute studies are imperative in order to pick the range of doses to be used in the 90-day range-finding study Note Existing screening studies on

acrylates could be deemed adequate for screening purposes if it can be shown that an MTD had been achieved according to the criteria to be established in the future EPA workshop ]

- In the 2-year bioassay, the consensus was to use <u>two doses</u> (plus a control)
  - One dose should be the MTD, based most likely on irritation (but on systemic toxicity if no irritation occurred in the dose-finding study)
  - The other dose should ideally be the highest one that induces no visual evidence of irritation in the dose-finding study
- Since only one sex and two doses are to be used, it was agreed that the number of animals should not be less than <u>50/group</u>
- The consensus was that full <u>histopathology</u> should be done at the top dose of the <u>90-day study</u> to identify possible target organs For the <u>limited bioassay</u> it was recommended that the following be considered for histopathologic examination
  - all parenchymal organs
  - skin from the site of application, as well as untreated skin
  - the "20 minimum tissues" listed by NTP's McConnell in Toxicologic Pathology, <u>11</u> 60 (1983)
  - any gross lesions

It was pointed out that small sections can be taken from most organs (with the exception of the CNS), that more than one tissue can be put in a block and more than one section can be put on a slide (approximately 6 mouse sections can fit on one slide)