UNCLASSIFIED

LIMITED TO
DEPARTMENTS OF DEFENCE
CAN, UK, US AND AUST, AND
TO CANADIAN DEFENCE
CONTRACTORS

#103474

# DRES

# CONTRACT REPORT 50-91

## CLINICAL RESEARCH STUDY ON SKIN SENSITIVITY TO A NEW REACTIVE SKIN DECONTAMINATION LOTION (RSDL) REF: DSS FILE 017SV.W8477-0-SC17/A A 21 DAY CUMULATIVE IRRITATION STUDY

## FINAL REPORT

Pharmaceutical Innovations

Winnipeg, Manitoba


September 1991

DEFENCE RESEARCH ESTABLISHMENT SUFFIELD, RALSTON, ALBERTA



UNCLASSIFIED

91-0479

CR-50-91
C.1

# CLINICAL RESEARCH STUDY

## ON SKIN SENSITIVITY TO A NEW REACTIVE SKIN DECONTAMINATION LOTION (RSDL)
## REF: DSS file 017SV.W8477-0-SC17/A

## A 21 DAY CUMULATIVE IRRITATION STUDY

## FINAL REPORT

Pharmaceutical Innovations
351 Tache Ave
Winnipeg, MB
R2H 2A6
Phone (204) 235-3450
FAX (204) 231-1918

# STATEMENT OF WORK

## CLINICAL RESEARCH

## STUDY

## 21 DAY CUMULATIVE IRRITATION STUDY

## SPONSOR

DEPARTMENT OF NATIONAL DEFENCE
OTTAWA, ONTARIO

J Harfield Purdon

SCIENTIFIC AUTHORITY: [signature] DATE: 2 Oct 90

# TABLE OF CONTENTS

# STATEMENT OF WORK

## 1. STUDY DESCRIPTION

This clinical safety trial is a 21-day occluded, randomized cumulative irritation trial of a reactive skin decontaminant lotion (RSDL) and the RSDL vehicle in 30 volunteers. After a 2 week rest subjects will receive a challenge test of each formulation to assess if sensitization occurs.

## 2. STATEMENT OF INVESTIGATION

### A. Study Sites

Study will be conducted at the clinical facilities of the contractor.

### B. Investigators

The principal investigator will be specified in the contract.

### C. Statement of Investigator

Investigator endorsement and participation in the study described herein includes acceptance and compliance with the conditions listed in the "Statement of Investigators".

## 3. ETHICS

### A. Regulations: Protection of Human Subjects and Provision of Institutional Review

In performing this investigation both the investigator and sponsor endorse, as a minimum, rules stated by the Federal code of Regulations Title 21, the Declaration of Helsinki, and the Medical Research Council of Canada (1987) regarding the protection of human patients and the provisions for Institutional Review Board (IRB) approval prior to the initiation of the study.

### B. Informed Consent

Written informed consent will be obtained from all patients, participating in this trial. The form and content of this document must be approved by the Institutional Review Board prior to its use.

C. Subject Release From Study

All patients are free to withdraw from participation in this study at any time and for whatever reason, specified or unspecified, and without prejudice.

## 4. BACKGROUND AND RATIONALE

The Department of National Defence has developed a lotion to be topically applied to the skin for use as a decontaminant against chemical agents such as mustard gas and nerve gases used in chemical warfare. The lotion is comprised of a 1.25 molal solution of potassium - 2,3 - butanedione monoximate (KBDO) in polyethylene glycol monomethyl ether 550 and 10% water.

A study in which KBDO was applied to a depilated area on the back of guinea pigs for 24 hours found the product to be non-irritating. No human irritation trials have been carried out.

The purpose of the present study is to evaluate the irritancy potential in man of KBDO and the base vehicle. The study is a 21-day occluded, double-blind, randomized cumulative irritation study in 30 volunteers. After a 2 week rest, subjects will receive a challenge test of each drug to access if sensitization occurs.

## 5. OBJECTIVES

To determine the skin irritation and sensitization of KBDO and the KBDO vehicle on healthy human skin.

## 6. CLINICAL SUPPLIES

A. Dosage Forms

The products and vehicle will be provided by the Department of National Defence, Defence Research Establishment Suffield, Ralston, Alberta.

B. Dosing

Products will be applied to each subject at pre-determined, randomized sites on the backs of 30 healthy male or female volunteers.

The products will be applied five days weekly for 21 days on the same site. Patches will not be re-applied on weekends or holidays, but the last patch applied will remain in place during these periods. Each test formulation will be occluded with a polypropylene chamber and covered with paper or Scanpore tape.

C. Packaging and Labelling of Clinical Supplies

All containers will be labelled with:

Study Number
Investigator's Name
Sample Number
Lot Number
Caution: Caution: Investigational Drug, For Use by Qualified Investigators Only

D. Blinding Safeguards

Formulations will be applied to pre-determined, randomized site locations on the backs of subjects by medical staff who will not be involved in the reading of the sites. Reading of irritation at each site of application will be carried out by separate medical personnel on a blinded basis. Subjects will also be blinded as to formulation location.

E. Drug Accountability

All drug supplies for this study must be stored in an area free of environmental extremes and with restricted access. Inventory checks will be conducted of the drug supplies at the study site from time to time and a drug receipt form will be signed by the investigator and placed in his/her files.

The investigator upon dispensing medication must record that information on a Drug Inventory and Dispensing Record Form to allow future accounting checks. A drug facility inspection will be conducted at appropriate time intervals throughout the clinical investigation. Any significant discrepancy and/or deficiency must be accounted for by the Principal Investigator on the Drug Inventory and Dispensing Record Form. At the conclusion of the study, the Principal Investigator must return all unused medication. Empty containers may be destroyed only on written authorization or returned.

## 7. PATIENT DEFINITION

Patients (normal subjects) must meet all of the inclusion criteria and none of the exclusion criteria. Subjects will be recruited by advertising or other means suitable for obtaining potentially compliant healthy volunteers.

### A. Number and Type

A total of 30 healthy volunteers will be recruited. An evaluable subject is defined as one who completes the entire study.

### B. Inclusion Criteria

1. Males and females (non-pregnant) may participate.
2. Females must be screened for childbearing potential. If females are of childbearing potential, e.g. they have not had a hysterectomy or tubal ligation, they must have a negative pregnancy test immediately prior to entrance into the study and be using an effective method of birth control.
3. Over 18 years of age.
4. Good general health.
5. Voluntary signing of Informed Consent statement prior to participation in the study.

### C. Exclusion Criteria

1. Dark skinned subjects, at the investigator's discretion.
2. Subjects who have significant skin disease, eg. acne, psoriasis.
3. Subjects who have participated in a patch test panel within 30 days prior to study initiation.
4. Subjects using any topical of chronic systemic medications.

### D. Subject Selection Checklist

The investigator will complete and sign the Subject Selection Checklist prior to application of the test materials.

### E. Concomitant Medications

Subjects must not be receiving any topical or chronic systemic medications. Subjects will be allowed to bathe or shower and use a non-medicated soap of their choice, but instruction to keep the test area dry. All medications

given to the subject during the course of the study, excluding the study drug, but including those medications given in treatment of adverse reactions will be recorded on the concomitant medication record. The drug name, dose, dosing frequency, and times(s) (24 hour clock) of administration are to be recorded. The indication(s) for each medication shall be noted as well as the possible effects of the agent on the subject's status.

F. Dropouts

Any dropouts from the study must be adequately described in the case report form.

8. STUDY PLAN

A. Study Design

This is a 21-day cumulative skin irritation study with a challenge test in healthy volunteers of 2 test articles applied to random sites on the backs of the subjects with evaluations carried out on a blind basis.

B. Randomization

Products will be randomly assigned to 2 pre-determined sites. Randomization codes will be provided to the clinic staff who will apply the materials, but not to the subjects or staff who are to grade the sites for irritation.

9. PROCEDURE

The 2 test materials will be applied to the backs of subjects using typical use concentrations (approximately 200 mg per patch) and covered for 24 hours with an occlusive and polypropylene chamber covered with paper or Scanpore tape. The application sites will be randomized among subjects by the investigator. Applications are repeated with the same test material and type of patch to the same site Monday through Friday, leaving the patches in place over the weekends, for a maximum of 21 days. This is a total of 15 applications over a three week period. Daily readings (Monday through Friday) of test case report form.

Test sites will be graded on the following basis:

0 = no visible reaction (negative)
0.5 = equivocal response (+)
1 = erythema
2 = erythema and induration
3 = erythema, induration and vesicles
4 = bullae

If a skin reaction >2 develops, it is the investigator's prerogative to discontinue application of the test material and that site will be graded a 4 reaction for each day remaining in the study. The readings must be made by the same person throughout the course of the study.

After a 2 week rest period each subject will be re-challenged with a 48 hour occluded test of each test item and evaluated for irritation at 48 and 96 hours after patch removal.

## 10. STUDY MANAGEMENT

### A. Potential Risks

Potential risks from the procedures to be performed are minimal. Patients may experience irritation, itching or reversible papule/pustule formation at the site(s) of application.

### B. Early Study Termination

If a patient is discontinued early from the study, the reason for termination must be provided, and entered on the appropriate Case Report Form.

Patients should be discontinued, or at least individual products discontinued, if severe irritation develops.

### C. Monitoring

A Department of National Defence or DND appointed representative will be allowed to conduct site visits to the investigational facilities for the purpose of monitoring the various aspects of the study.

### D. Case Report Forms

Case Report Forms will be printed on NCR (no carbon required) paper to permit multiple copies. The bottom copy (buff) is to be retained by the investigator for his/her files.

Data collected on each patient will be recorded on the appropriate case report form provided. The investigator will be responsible for ensuring that all blank data spaces on each form are filled in. If certain data are not available, the investigator will enter "N.A." in the appropriate space.

Completed case report forms are to be signed by the Principal Investigator in the appropriate location and submitted to the contractor clinical monitor.

The clinical Monitor will review all case report forms, evaluate them for completeness with reference to other records as necessary, and return all forms with missing data and/or errors to the investigator for correction.

Changes/additions to data entered on original case report forms must be made in the following manner: the original entry will be lined out (not erased or "whited out") so as to leave it still legible. The correction will be entered in black ink, initialled and dated by the person making the correction.

E. Adverse Experiences

In the event of a serious adverse reaction, Department of National Defence must be notified immediately by telephone. The experience must be completely described in the case report.

All adverse signs and symptoms which occur during or following the course of drug administration must be reported in detail on the subject's case report. This description is to include the nature of the sign or symptom, time of onset in relation to drug administration, duration, severity, possible relationship to drug, required therapy, and outcome.

When an emergency occurs that required departure from the protocol for an individual subject, it will be only for that subject. The investigator or other physician in attendance during the emergency will contact DND as soon as possible by telephone.

Such contact with DND will be made as soon as possible to permit a decision as to whether or not the subject (for whom the departure from the protocol was effected) is to continue in the study.

The case report will describe the departure from the protocol, reason(s) for it, individual contacted at DND, and date of contact.

F. Investigator's Reporting Obligations

The investigator agrees to furnish DND with complete subject identification on the Confidential Subject Follow-Up Form which will be used for purposes of long-term follow-up studies if needed. These will be filed at Department of National Defence under adequate security with accessibility restricted to the Study Director and will be treated with strict adherence to professional standards of confidentiality.

Otherwise, all reports and communications relating to subjects in the study will identify each subject only by the subject's study number, first three letters of the last name and DND study number.

The following will be submitted to DND:

1. Prior to initiation of the study and receipt of clinical drug supplies:

   (a) Statement of Investigator and a signed, dated up-to-date curriculum vitae for the principal investigator, as well as curriculum vitae for any sub or co-investigators who will have responsibility for dispensing study medications;

   (b) A copy of the approval letter or notice by the Institutional Review Board. The approval letter or notice must contain the date of the meeting, the protocol number, title of the study, a summary of any appropriate discussion, and requirements for periodic review of this protocol. It is also necessary to submit a list of institutional affiliations and occupations of members of the IRB; and

   (c) A sample copy of the informed consent form that is to be used in this study to elicit and record the subject's consent, which has been approved by the IRB in compliance with HPB guidelines and U.S. Food and Drug Administration regulations.

2. Forms in the case report with notation of who is to complete each one any other special instructions needed.

This includes:

(a) Subject Section Checklist - to be completed by the investigator prior to application of test materials.

(b) Patch Test Data Sheet - to be supplied and completed by the investigator on a daily basis at the time the test sites are read.

(c) Concomitant Medications CRF.

(d) Adverse Reactions CRF.

(e) Study Termination Record.

All case report forms will be completed and signed by the Investigator. All case report form entries must be in black ink. The Investigator must review all entries for completeness and correctness, then sign each individual form. When changes or corrections are made on the case report form, the Investigator or co/sub-investigator must draw a line through the error, initial and date the correction.

6. Amendments to the Protocol

Changes in any portion of the protocol after signatures of agreement are obtained must be documented in the form of an amendment, and signed by the clinical monitor and the investigator(s).

All amendments must be submitted to the Institutional Review Board (IRB) and approved prior to the implementation. If the change is minor, the IRB Chairperson alone may approve it.

The only circumstances in which an amendment may be initiated without IRB approval is to eliminate apparent immediate hazards to the patients. However, the investigator must notify the IRB within ten working days after implementation.

All amendments to the protocol which result in a substantial change in study design or procedures will be submitted to DND for approval.

## 11. STUDY EVALUATION

Each patch test site will be scored daily on a 0-4 scale. Daily and study mean scores (+SD) will be calculated for each test article. The vehicle control is included to assist in determining the cause of skin irritation should it occur. It is generally possible at the completion of the study to determine if each test product is more, less or equally as irritating.

## 12. APPROVAL SIGNATURES

Investigator:

_____ _____
Date

_____ _____
Date

Only the above may validate case report forms by their signature. Investigators who wish to delegate routine signing to a responsible person must provide the sponsor with a written request to this end, together with a specimen signature.

Department of National Defence

_____ _____
Date

TABLE OF CONTENTS

CLINICAL RESEARCH STUDY
21 DAY CUMULATIVE IRRITATION STUDY

Introduction:

A lotion has been developed for use as a topical decontaminant against chemical agents such as mustard and nerve gases. The lotion consists of a 1.25 M solution of potassium 2,3-butanedione monoximate (KBDO) in polyethylene glycol monomethyl ether 550 and 10% water.

Testing of the product for 24 hours on a depilated area on the back of guinea pigs showed that the product was non-irritating.

The purpose of the present study was to evaluate the irritancy potential in man of KBDO and the base vehicle. The study was a 21 day occluded, double-blind, randomized trial using 32 volunteers. After a two week rest, the subjects received a challenge test of each drug to assess if sensitization had occurred.

Study Design:

The study involved the testing of KBDO lotion, lotion vehicle and a blank dressing on three randomly chosen sites on 32 healthy volunteers. The backs of the subjects were divided into six approximately equal areas bounded as follows:

A. Trapezius, L spinal process, T6
B. Trapezius, R spinal process, T6
C. T6, L spinal process, T11
D. T6, R spinal process, T11
E. T11, L spinal process, iliac crest
F. T11, R spinal process, iliac crest

For each study subject, three areas were randomly selected, one for KBDO lotion, one for lotion vehicle, and one for a blank dressing. A 25 ug quantity of test lotion and of vehicle were applied to the designated sites respectively, and covered with a polyethylene disk (diameter 3.5 cm) held in place with an occlusive dressing (Opsite). Applications were repeated with the same test material and type of patch to the same site on a daily basis from Monday to Friday. The

patches were left in place over the weekends. The sites were examined prior to each re-application and graded according to the following grade scale:

| GRADE | SITE APPEARANCE |
|---|---|
| 0.0 | no visible reaction (-) |
| 0.5 | equivocal response (+) |
| 1.0 | erythema |
| 2.0 | erythema and induration |
| 3.0 | erythema and induration and vesicles |
| 4.0 | bullae |

Should a skin reaction of grade 2.0 or greater be seen, the study subject would be examined by one of the study physicians to determine the appropriate course of action.

Application of the product and patch were performed by one of the principal investigators (DC) and the study nurse while the evaluation of the site was performed by the other principal investigator (MT). At the time of site evaluation a permanent coded photographic record was made.

After a rest of two weeks each subject was challenged by re-application of each test substance to the originally randomized sites for a 48 hour period. The application sites were evaluated at the end of the 48 hour exposure then again 48 hours after removal of the patches.

Study subjects:

A total of thirty-two (32) volunteers for the study were recruited from staff of the St. Boniface General Hospital. The study population consisted of eighteen (18) males and fourteen (14) females between the ages of 20 to 54. Immediately prior to entry into the study all subjects read and signed an informed consent form previously approved by the University of Manitoba Committee for the use of Human Subjects in Research. The signed consent forms are included in the Patient Data Files. Blood samples were drawn for serum creatinine, urea, gamma-GT and a complete blood count. These tests were repeated on day 21 of the study. All female study subjects of childbearing potential had a pregnancy test (b-HCG) performed prior to the study.

Subject Exclusions:

The following subjects were not to be entered into the study:

1) Subjects who have significant skin disease or a condition which would impair the visual evaluation of reaction

2) Individuals who have participated as subjects in any clinical trial within 30 days prior to study initiation

3) Subjects using any topical or chronic systemic medications excluding oral contraceptives

Study Population:

The study population consisted of 18 males and 14 females for a total of 32 subjects. The mean age of the population was 32.9 years; the mean height was 168.29 cm; and the mean weight was 71.5 Kg. The details of these parameters are presented in Table 1.

Subject #003 was withdrawn from the study on day 3 due to skin breakdown from the Opsite dressings and subjects #024 and #026 declined to participate in the study after reading and discussing the informed consent.

KBDO STUDY -- Subject Data

| Subject | Age (y) | Height (cm) | Weight (Kg) | Sex |
|---|---|---|---|---|
| 001 | 22 | 180.00 | 77.3 | M |
| 002 | 27 | 172.50 | 68.2 | M |
| 004 | 30 | 173.75 | 84.1 | M |
| 005 | 47 | 160.00 | 63.3 | F |
| 006 | 30 | 185.00 | 75.0 | M |
| 007 | 28 | 160.00 | 100.0 | F |
| 008 | 35 | 157.50 | 52.3 | F |
| 009 | 34 | 181.30 | 77.3 | M |
| 010 | 25 | 170.00 | 79.6 | M |
| 011 | 45 | 152.50 | 56.8 | F |
| 012 | 20 | 172.50 | 62.7 | F |
| 013 | 21 | 175.00 | 65.9 | M |
| 014 | 25 | 175.00 | 65.9 | M |
| 015 | 23 | 150.70 | 63.6 | F |
| 016 | 41 | 162.50 | 60.0 | F |
| 017 | 51 | 160.00 | 72.7 | F |
| 018 | 23 | 160.00 | 47.3 | F |
| 019 | 52 | 170.00 | 79.0 | M |
| 020 | 23 | 162.50 | 62.3 | F |
| 021 | 34 | 164.00 | 68.0 | M |
| 022 | 22 | 180.00 | 77.3 | M |
| 023 | 35 | 170.00 | 65.9 | M |
| 025 | 29 | 180.00 | 80.9 | M |
| 027 | 29 | 175.00 | 88.6 | M |
| 028 | 41 | 180.00 | 93.2 | M |
| 029 | 33 | 172.50 | 61.4 | F |
| 030 | 32 | 147.50 | 52.3 | F |
| 031 | 29 | 162.50 | 65.9 | F |
| 032 | 54 | 162.50 | 90.9 | F |
| 033 | 28 | 170.00 | 56.8 | M |
| 034 | 45 | 180.00 | 80.0 | M |
| 035 | 41 | 160.60 | 94.6 | M |
| Mean | 32.9 | 168.29 | 71.5 | |
| Std Dev | 9.75 | 9.77 | 13.28 | |

n = 32

Table 1

Lotion Application and Dosage:

Three sites on the backs of the subjects were selected on a random basis as described under Study Design. One site was for KBDO lotion, one for lotion vehicle and one for a blank dressing.

The mean total dose of KBDO lotion applied per subject for the 21 day period was 380 mg and the mean individual dose was 27.9 mg. The dose was applied, covered with a 3.5 cm polyethylene disk and the whole then covered with an occlusive dressing. The dose applied was calculated by weighing the lotion container before and after application with the difference in weight being taken as the dose. Records of the weighing are contained in the Patient Files and a summary of doses and sites are presented in Table 2.

KBDO STUDY -- Lotion dosages and sites

| Subject | Total Dose(mg) | Average Dose(mg) | Test | Control | Blank |
|---|---|---|---|---|---|
| 001 | 430 | 33.1 | E | B | A |
| 002 | 390 | 30.0 | C | E | B |
| 004 | 410 | 29.3 | A | E | D |
| 005 | 350 | 25.0 | F | A | E |
| 006 | 370 | 26.4 | C | A | F |
| 007 | 340 | 26.2 | D | E | A |
| 008 | 340 | 24.3 | C | B | D |
| 009 | 370 | 26.4 | F | D | E |
| 010 | 470 | 36.2 | A | C | F |
| 011 | 370 | 28.5 | E | F | C |
| 012 | 410 | 29.3 | D | C | B |
| 013 | 350 | 25.0 | E | C | A |
| 014 | 380 | 27.1 | B | C | D |
| 015 | 330 | 25.4 | C | D | B |
| 016 | 340 | 26.2 | C | F | E |
| 017 | 460 | 32.9 | A | F | C |
| 018 | 400 | 28.6 | A | D | F |
| 019 | 300 | 21.4 | B | F | A |
| 020 | 420 | 35.0 | A | B | C |
| 021 | 400 | 28.6 | B | A | D |
| 022 | 470 | 33.6 | B | E | F |
| 023 | 370 | 26.4 | B | D | E |
| 025 | 350 | 25.0 | D | F | B |
| 027 | 390 | 27.9 | E | A | D |
| 028 | 320 | 22.9 | E | D | F |
| 029 | 280 | 20.0 | F | C | E |
| 030 | 350 | 25.0 | D | B | A |
| 031 | 330 | 23.6 | B | D | C |
| 032 | 350 | 26.9 | F | C | A |
| 033 | 370 | 26.4 | B | E | F |
| 034 | 400 | 30.8 | C | B | A |
| 035 | 550 | 39.3 | C | E | D |
| Mean | 380 | 27.9 | | | |
| Std dev | 54.9 | 4.29 | | | |

n = 32

Table 2

Results:

(a) Serum biochemistry/hematology and pregnancy testing:

On study days 0 and 21, blood samples were taken for measurement of serum creatinine, urea, gamma-GT and CBC. The measured values for the two days are presented in Tables 3 and 4. The subjects were all in good health and the values obtained were within normal laboratory values.

None of the female subjects were pregnant at the start of the study as shown by b-HCG urine testing. All female subjects were advised both verbally and in the informed consent to avoid becoming pregnant during the time of the study.

KBDO STUDY -- Day 0 Serum biochemistry and hematology

| Subject | WBC | RBC | HgB | PLT | EOS | Urea | Creat | GGT |
|---|---|---|---|---|---|---|---|---|
| 001 | 8.7 | 5.64 | 171 | 290 | 3 | 4.8 | 107 | 29 |
| 002 | 5.3 | 4.50 | 131 | 235 | 2 | 8.6 | 85 | 10 |
| 004 | 5.9 | 4.69 | 144 | 334 | 4 | 6.2 | 121 | 20 |
| 005 | 6.7 | 4.20 | 129 | 313 | 2 | 6.6 | 94 | 11 |
| 006 | 9.2 | 4.48 | 138 | 298 | 5 | 4.6 | 89 | 11 |
| 007 | 6.3 | 4.86 | 139 | 221 | 4 | 2.8 | 82 | 12 |
| 008 | 6.0 | 4.15 | 131 | 226 | 4 | 5.2 | 86 | 12 |
| 009 | 5.4 | 4.80 | 148 | 230 | 2 | 5.6 | 101 | 10 |
| 010 | 6.1 | 5.57 | 174 | 215 | 3 | 4.8 | 114 | 14 |
| 011 | 8.7 | 4.86 | 151 | 161 | 3 | 4.1 | 85 | 12 |
| 012 | 6.8 | 3.97 | 131 | 297 | 2 | 5.3 | 86 | 9 |
| 013 | 8.3 | 5.36 | 165 | 260 | 2 | 5.2 | 102 | 13 |
| 014 | 6.7 | 5.07 | 156 | 247 | 1 | 4.6 | 105 | 11 |
| 015 | 7.4 | 4.33 | 130 | 297 | 1 | 3.6 | 88 | 21 |
| 016 | 6.8 | 4.13 | 125 | 254 | 2 | 5.2 | 82 | 9 |
| 017 | 7.9 | 4.93 | 140 | 294 | 1 | 4.4 | 82 | 19 |
| 018 | 6.7 | 4.65 | 139 | 212 | 2 | 3.7 | 87 | 20 |
| 019 | 5.8 | 4.69 | 150 | 202 | 4 | 4.1 | 96 | 21 |
| 020 | 7.1 | 4.47 | 136 | 304 | 4 | 3.6 | 94 | 13 |
| 021 | 6.8 | 4.68 | 144 | 203 | 2 | 6.3 | 97 | 16 |
| 022 | 9.7 | 4.47 | 145 | 297 | 1 | 5.7 | 105 | 23 |
| 023 | 6.7 | 5.22 | 151 | 276 | 2 | 5.0 | 112 | 37 |
| 025 | 6.2 | 4.82 | 151 | 217 | 3 | 5.8 | 119 | 22 |
| 027 | 6.5 | 4.89 | 150 | 262 | 2 | 4.6 | 106 | 22 |
| 028 | 6.5 | 4.34 | 141 | 291 | 2 | 6.4 | 106 | 22 |
| 029 | 5.4 | 4.28 | 133 | 288 | 2 | 4.3 | 81 | 14 |
| 030 | 5.9 | 4.21 | 136 | 240 | 1 | 4.4 | 79 | 19 |
| 031 | 7.0 | 4.62 | 136 | 257 | 2 | 3.1 | 77 | 13 |
| 032 | 5.5 | 4.19 | 130 | 249 | 3 | 3.4 | 94 | 38 |
| 033 | 4.6 | 4.44 | 143 | 277 | 3 | 4.1 | 97 | 30 |
| 034 | 8.0 | 4.89 | 152 | 234 | 2 | 4.8 | 101 | 11 |
| 035 | 3.5 | 5.07 | 160 | 118 | 1 | 6.7 | 104 | 16 |
| Mean | 6.7 | 4.67 | 144 | 253 | 2.4 | 4.9 | 96 | 17.5 |
| Std dev | 1.33 | 0.42 | 12.3 | 46.5 | 1.07 | 1.22 | 12.0 | 7.7 |

n = 32

Table 3

KBDO STUDY -- Day 21 Serum biochemistry and hematology

| Subject | WBC | RBC | HgB | PLT | EOS | Urea | Creat | GGT |
|---|---|---|---|---|---|---|---|---|
| 001 | 8.4 | 4.96 | 151 | 276 | 2 | 5.3 | 98 | 24 |
| 002 | 4.7 | 4.60 | 136 | 233 | 1 | 4.2 | 86 | 11 |
| 004 | 5.8 | 4.64 | 143 | 336 | 4 | 5.3 | 103 | 19 |
| 005 | 8.3 | 4.42 | 136 | 357 | 2 | 4.9 | 81 | 12 |
| 006 | 9.5 | 4.34 | 133 | 348 | 4 | 4.9 | 81 | 12 |
| 007 | 7.3 | 4.69 | 133 | 203 | 5 | 3.6 | 78 | 11 |
| 008 | 6.4 | 3.99 | 126 | 229 | 5 | 5.1 | 84 | 11 |
| 009 | 5.2 | 4.76 | 149 | 240 | 3 | 5.6 | 91 | 11 |
| 010 | 5.2 | 5.32 | 168 | 215 | 3 | 4.8 | 105 | 14 |
| 011 | 10.2 | 4.71 | 145 | 169 | 11 | 3.6 | 81 | 13 |
| 012 | 7.9 | 4.12 | 134 | 311 | 2 | 5.5 | 79 | 9 |
| 013 | 9.0 | 5.08 | 158 | 227 | 2 | 5.5 | 93 | 15 |
| 014 | 5.4 | 5.00 | 152 | 219 | 2 | 4.2 | 104 | 11 |
| 015 | 6.6 | 4.32 | 131 | 316 | 2 | 3.5 | 81 | 22 |
| 016 | 7.1 | 4.25 | 128 | 205 | 1 | 4.6 | 74 | 10 |
| 017 | 6.3 | 5.01 | 145 | 277 | 1 | 3.8 | 73 | 21 |
| 018 | 6.1 | 4.30 | 130 | 177 | 2 | 4.5 | 85 | 20 |
| 019 | 5.9 | 4.69 | 150 | 200 | 4 | 3.8 | 95 | 21 |
| 020 | 6.8 | 4.33 | 131 | 245 | 4 | 3.5 | 89 | 13 |
| 021 | 7.2 | 4.61 | 140 | 220 | 2 | 5.8 | 92 | 15 |
| 022 | 9.0 | 4.45 | 144 | 271 | 1 | 5.4 | 99 | 22 |
| 023 | 6.2 | 5.34 | 156 | 301 | 3 | 4.6 | 107 | 31 |
| 025 | 7.6 | 4.96 | 154 | 205 | 4 | 6.4 | 117 | 20 |
| 027 | 5.5 | 5.00 | 153 | 227 | 2 | 5.0 | 99 | 20 |
| 028 | 6.4 | 4.35 | 141 | 283 | 2 | 7.3 | 86 | 21 |
| 029 | 5.0 | 4.14 | 130 | 258 | 3 | 4.2 | 78 | 13 |
| 030 | 6.7 | 4.39 | 140 | 188 | 1 | 3.9 | 72 | 12 |
| 031 | 6.1 | 4.68 | 138 | 228 | 1 | 3.9 | 72 | 12 |
| 032 | 7.5 | 4.13 | 129 | 268 | 3 | 5.2 | 85 | 42 |
| 033 | 4.8 | 4.84 | 156 | 267 | 3 | 4.5 | 109 | 31 |
| 034 | 7.4 | 4.88 | 150 | 222 | 3 | 4.7 | 96 | 11 |
| 035 | 3.6 | 5.05 | 159 | 117 | 1 | 5.7 | 93 | 16 |
| Mean | 6.7 | 4.64 | 143 | 245 | 2.7 | 4.8 | 89.6 | 17 |
| Std dev | 1.51 | 0.36 | 10.9 | 54.0 | 1.9 | 0.89 | 11.8 | 7.4 |

n = 32

Table 4

(b) Skin sensitivity testing - 21 day cumulative study:

Skin Reactions

| Subject | Study Day | Site/Grade Test | Control | Blank |
|---|---|---|---|---|
| 002 | 6 | - | 0.5 | - |
| | 7 | - | 0.5 | - |
| | 8 | - | 0.5 | - |
| 006 | 1 | - | - | 0.5 |
| | 3 | - | - | 0.5 |
| 007 | 3 | - | - | 0.5 |
| | 7 | - | - | 0.5 |
| | 8 | - | - | 0.5 |
| | 9 | - | - | 0.5 |
| | 10 | - | - | 0.5 |
| 008 | 1 | - | 0.5 | 0.5 |
| | 3 | - | - | 0.5 |
| | 6 | - | - | 0.5 |
| | 7 | - | - | 0.5 |
| | 8 | - | - | 0.5 |
| | 9 | - | - | 0.5 |
| | 10 | - | - | 0.5 |
| | 20 | - | - | 0.5 |
| 009 | 6 | - | 0.5 | - |
| | 7 | - | 0.5 | - |
| | 8 | - | 0.5 | - |
| | 9 | - | 0.5 | - |
| | 10 | - | 0.5 | - |
| | 20 | 0.5 | - | - |
| 014 | 16 | 0.5 | - | - |
| 015 | 20 | - | - | 0.5 |
| 018 | 6 | - | - | 0.5 |

Skin Reactions (Cont'd)

| Subject | Study Day | Site/Grade Test | Control | Blank |
|---|---|---|---|---|
| 022 | 1 | - | - | 0.5 |
| 025 | 1 | - | 0.5 | - |
| | 9 | - | - | 0.5 |
| 027 | 2 | 0.5 | - | 0.5 |
| | 3 | 0.5 | - | - |
| 030 | 1 | 0.5 | - | 0.5 |
| | 2 | - | - | 0.5 |
| | 3 | - | - | 0.5 |
| | 4 | - | - | 0.5 |
| | 9 | - | - | 0.5 |
| | 10 | - | - | 0.5 |
| 033 | 7 | - | - | 0.5 |
| | 8 | - | - | 0.5 |

Table 5

Anatomical sites and frequency of reaction: *

| Subject | Test | Control | Blank ** |
|---|---|---|---|
| 002 | - | E ( 3) | - |
| 006 | - | - | F ( 2) |
| 007 | - | - | A ( 5) |
| 008 | - | B ( 1) | D ( 8) |
| 009 | F (1) | D ( 5) | - |
| 014 | B (1) | - | - |
| 015 | - | - | B ( 1) |
| 018 | - | - | F ( 1) |
| 022 | - | - | F ( 1) |
| 025 | - | F ( 1) | B ( 1) |
| 027 | E (2) | - | D ( 1) |
| 030 | D (1) | - | A ( 6) |
| 033 | - | - | F ( 2) |
| Totals 13 | 4 (5) | 4 (10) | 10 (28) |

* All reactions were of grade 0.5
** Anatomical site with number of positive observations in parenthesis

Table 6

(c) Challenge testing:

After a two week rest period, the subjects had the testing procedure repeated. None of the sites on any of the patients showed any reaction at the 48 hour observation and at the end of the 96 hour observation period all sites appeared normal with healthy, clear skin.

Discussion:

A total of 32 subjects participated in this study. Blood samples drawn on day 0 and day 21 of the study for serum biochemistry and hematology were within limits for normal values. The group values for each of the measured parameters were unchanged ($p<0.05$) over the course of the study as shown by analysis of variance (ANOVA). Subject #011 showed an increase in EOS and WBC possibly secondary to seasonal rhinitis, the symptoms of which appeared midway through the study.

Only very minor skin irritation was seen during the study (grade 0.5). A total of 13 subjects showed any skin irritation with 4 showing irritation at the test lotion site, 4 at the lotion control site and 10 at the blank dressing site. The total number of observations showing irritation at the test site was 5; at the control site 10 and at the blank dressing site 28. There appeared to be no one particular anatomical site which was more prone to irritation than another. Although there was little skin irritation at the sites under the polyethylene disks, there was skin irritation due to the removal of the adhesive occlusive dressings. This was probably a mechanical irritation due to the removal of the top skin layers by the adhesive. Initially, Opsite dressings were used and if irritation occurred from the adhesive, Mefix was substituted as the occlusive dressing. About 30% of the subjects ultimately required Mefix dressings and the dates when substitution occurred are recorded in the Patient Data Files. Generally if substitution was needed, it was apparent within the first week of the study and all sites for the subject were substituted. If substitution was needed, Mefix was used for the balance of the study. Mefix dressings appeared to be much less damaging to the skin and only one subject (#003) was withdrawn from the study due to skin breakdown from the occlusive dressings.

With the exception of one, none of the subjects complained of any unusual symptoms or malaise during the study. The one exception (subject #017) complained of a metallic taste and flushing about two hours after application of the test patches. This complaint was relatively consistent and although it suggested a possible systemic effect, the subject declined to withdraw from the study. A timed urine sample was obtained from the subject on day 21 and screened for 2,3-butanedione monoxime content using an HPLC method. The details of this investigation are detailed in Appendix A.

Although the lotion had a green-yellow color which was quite visible when first applied to the skin, the color was gone within the 24 hour observation period.

Each subject was challenged by application of the patches two weeks after completion of the 21 day cumulative study. None of the subjects showed any signs of skin irritation when examined at 48 hours after application and at the 96 hour examination, the skin appeared normal and clear.

Conclusion:

Based on the results of this study, the KBDO lotion tested appeared to cause no unusual irritation to the skin after a period of prolonged use and did not appear to cause any type of topical sensitivity. The dose applied did not cause any significant alteration of the serum hematology or biochemistry variables which were monitored. There is a possibility that the active ingredient in the lotion may be subject to transdermal absorption.

Appendix A

Preliminary urine screening for 2,3-butanedione monoxime after topical application of KBDO lotion

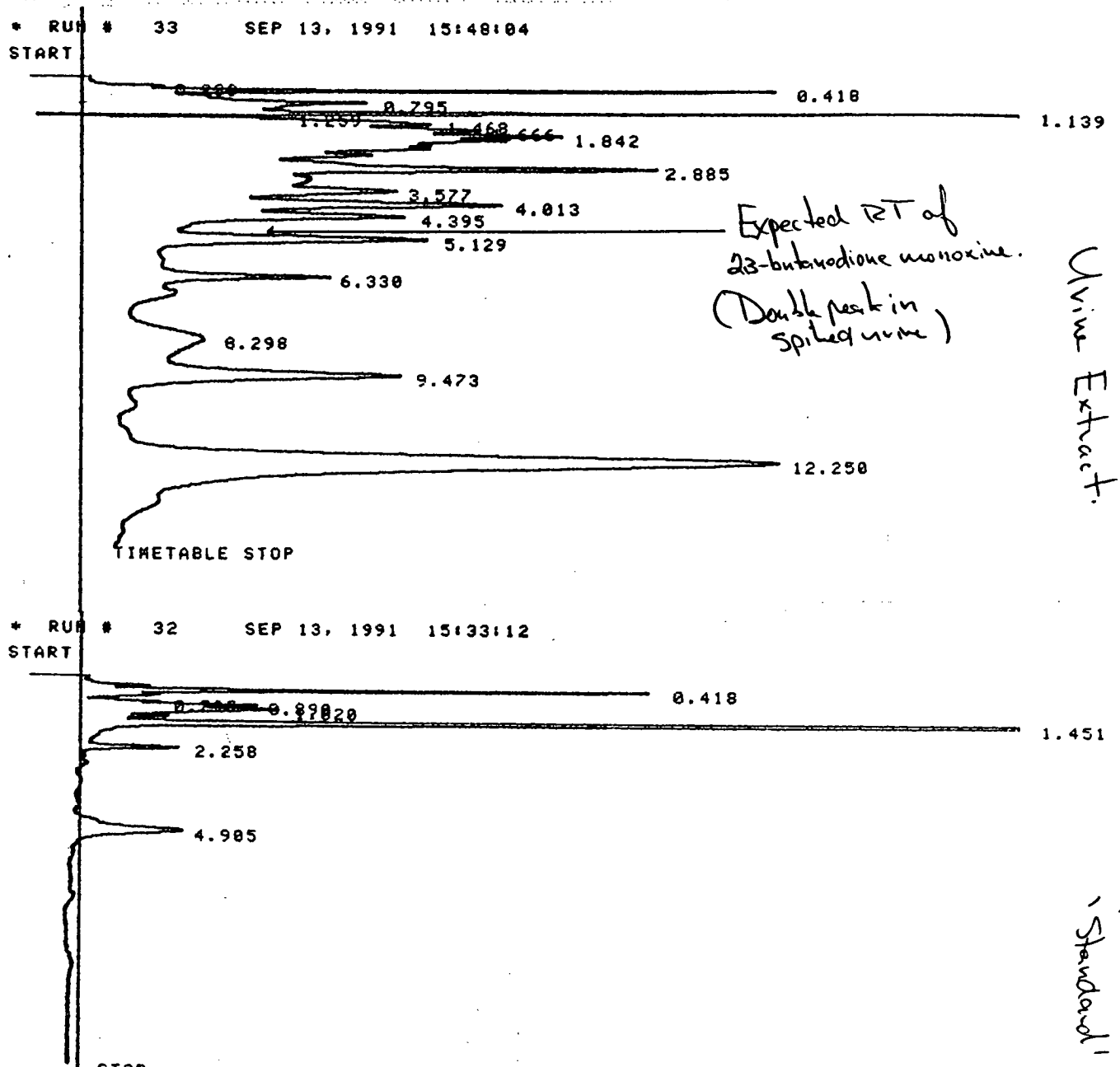
During the course of the 21 day cumulative skin sensitivity study of KBDO lotion, one subject (#017) complained of a metallic taste and a sensation of flushing about two hours after application of the lotion. This response was quite consistent over the entire course of the study and suggested that some of the active component may be dermally absorbed and exert some systemic effects.

On day 21 of the study, the subject emptied her bladder prior to application of the KBDO lotion and collected her urine for eight hours after KBDO application. Approximately 375 mL of urine was collected. A 200 mL aliquot was taken, acidified with 2 M HCl to pH 3.0 and extracted with three 50 mL portions of ether. The ether was removed under a stream of nitrogen and the residue was dissolved in 10 mL of methanol:water (65:35). The urine was examined for 2,3-butanedione monoxime by HPLC. The chromatographic system consisted of a 15 cm HiSep (shielded hydrophobic phase) column using a mobile phase of methanol:water (65:35) with a flow rate of 1.5 mL/min. Detection was by UV absorption at 227 nm. The HPLC method was provided by Farrington-Lockwood.

The following solutions were prepared : a standard solution consisting of 10 uL of KBDO lotion and 10 uL of 2 M HCl made to 10 mL with mobile phase and a blank solution consisting of 10 uL of lotion vehicle and 10 uL of 2 M HCl made to 10 mL with mobile phase. The test solution was the urine extract previously described and a 5 mL portion of the urine extract was spiked with 5 uL of KBDO lotion and 5 uL of 2 M HCl. A sample of the raw urine was also run.

A peak with a retention time of 4.9 minutes was seen in the standard solution and the spiked urine and assumed to represent the 2,3-butanedione monoxime. No corresponding peak was found in the blank, urine extract or raw urine sample.

The apparent absence of 2,3-butanedione monoxime in the urine does not dismiss the possibility of transdermal absorption of KBDO but does suggest that if it is absorbed, it is not excreted intact in the urine. Pending the outcome of other studies with KBDO lotion, this aspect may require further study.







RUN# 32 SEP 13, 1991 15:33:12

AREA%

| RT | AREA | TYPE | WIDTH | AREA% |
|---|---|---|---|---|
| 1.451 | 391462 | VB | .076 | 100.00000 |

TOTAL AREA= 391462
MUL FACTOR=1.0000E+00