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

received
March 28, 2005

March 17, 2005

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

CHEMINOVA A/S
1620 EYE STREET NW, SUITE 615
WASHINGTON, DC 20006-

original: Paul
copies: David M.
Kristian L.

Report of Analysis for Compliance with PR Notice 86-5

Thank you for your submittal of 17-MAR-05. Our staff has completed a preliminary analysis of the material. The results are provided as follows:

Your submittal was found to be in full compliance with the standards for submission of data contained in PR Notice 86-5. A copy of your bibliography is enclosed, annotated with Master Record ID's (MRIDs) assigned to each document submitted. Please use these numbers in all future references to these documents. Thank you for your cooperation. If you have any questions concerning this data submission, please raise them with the cognizant Product Manager, to whom the data have been released.

464976-00

CHEMINOVA

Cheminova, Inc
Washington Office
1620 Eye Street, N.W.
Suite 615
Washington, D.C. 20006

Phone: 202-463-1492
Fax: 202-463-1493

HAND DELIVERED

February 18, 2005

Ms. Stephanie Plummer
Chemical Review Manager - Dimethoate
Special Review and Reregistration Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
C/O Document Processing Desk (Room 266A)
1801 South Bell Street
Arlington, VA 22202

RE: Dimethoate Human Oral Metabolism Study

Dear Ms. Plummer:

On behalf of Cheminova A/S (Cheminova, EPA Company No. 4787), I am submitting three copies of the following report:

46497601 • Urinary excretion profile of Dimethoate and its metabolites after single oral administration of Dimethoate in male volunteers. Report prepared for the Dimethoate Task Force (DTF) by TNO Nutrition and Food Research Physiological Sciences Department. TNO Project No. 010.44162. Final report dated December 28, 2004.

The enclosed study was sponsored by the Dimethoate Task Force (DTF) in Europe (of which Cheminova is a member) to support the use of urine biomonitoring as means for determining internal dose received after exposure to dimethoate. In this study, six healthy adult male volunteers were given a single bolus dose of dimethoate at 0.03 mg/kg bw. Plasma and red blood cell (RBC) cholinesterase activity and the metabolism and urinary excretion patterns were evaluated in each volunteer. Results of the study demonstrated no cholinesterase inhibition in volunteers after a single oral dose of 0.03 mg/kg/day and that the metabolism and excretion profiles in rats and humans are substantially similar.

If you have any questions about the information provided in this letter, or if you need additional information, please feel free to call me at (202) 463-1490, or Paul Whatling at (202) 463-1491.

Sincerely,

Diane Allemang
Director of Regulatory Affairs
Cheminova, Inc.
EPA Agent for Cheminova A/S

Enclosure

c: Kristian Lystbæk, Cheminova A/S
David Menotti, Shaw Pittman
Paul Whatling, Cheminova, Inc.

STUDY TITLE

Urinary Excretion Profile of Dimethoate and its Metabolites after Single Oral Administration of Dimethoate in Male Volunteers.

DATA REQUIREMENT

Not Applicable

STUDY DIRECTOR/AUTHOR

W.J.A. Meuling, B.Sc., L. Roza, Ph.D.

STUDY COMPLETION DATE

December 28, 2004

PERFORMING LABORATORY/SPONSOR

Dimethoate Task Force (DTF)
Wotanstrasse 39, D-68305
Mannheim
Germany

LABORATORY PROJECT I.D.

V4802

SPONSOR/SUBMITTER

Cheminova A/S
(EPA Company No. 4787)
P.O. Box 9
DK-7620 Lemvig
Denmark

VOLUME 1 OF 2

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10(d)(1)(A), (B) or (C). This statement supersedes any other statement of confidentiality that may appear in this report.

Company: Cheminova A/S

Company Agent: Diane Allemang

Title: Director, Regulatory Affairs

Signature: Diane Allemang Date: 2/18/05

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

TNO Nutrition and Food Research

I, the undersigned, hereby declare that to the best of my knowledge this report constitutes a true and complete representation of the procedures followed and of the results obtained in this study by TNO Nutrition and Food Research. However, part of the study have been carried out by Huntingdon Life Sciences Ltd. UK (chemical analysis) and Diagnostisch Centrum SSDZ, Delft NL (cholinesterase measurements). There were no situations in this study which have affected the quality or integrity of the data The study was carried out under my overall supervision and conducted in accordance with the ICH Guideline for Good Clinical Practice (ICH topic E6, adopted 01-05 1996 and implemented 17-01-1997).

W.J.A. Meuling, BSc

Principal Investigator

28/12/04

Date / Signature

Approved by

p/a

J.P. Bijsten

(HEAD, DEPT physiological sciences)

Ms. A.F.M. Kardinaal, MSc, PhD

Head Operations Physiological Sciences Department

28/12/2004

Date / Signature

Sponsor/Submitter:

Diane Allemang

Director, Regulatory Affairs

Cheminova, Inc.

(U.S. Agent for Cheminova A/S)

2/18/05

Date

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DECLARATION AND SIGNATURE PAGES

AUTHENTICATION BY THE PRINCIPAL INVESTIGATOR

TNO Nutrition and Food Research

I, the undersigned, hereby declare that to the best of my knowledge this report constitutes a true and complete representation of the procedures followed and of the results obtained in this study by TNO Nutrition and Food Research. However, part of the study have been carried out by Huntingdon Life Sciences Ltd UK (chemical analysis) and Diagnostisch Centrum SSDZ, Delft NL (cholinesterase measurements). There were no situations in this study which have affected the quality or integrity of the data. The study was carried out under my overall supervision and conducted in accordance with the ICH Guideline for Good Clinical Practice (ICH topic E6, adopted 01-05 1996 and implemented 17-01-1997).

28/12/04

W.J.A. Meuling, BSc

Date / Signature

Principal Investigator

Approved by

pka

S.P. Bieten
(Head dept physiological sciences)

Ms. A.F.M. Kardinaal, MSc, PhD

28/12/2004

Date / Signature

Head Operations Physiological Sciences Department

STATEMENT BY THE MEDICAL INVESTIGATOR

I, the undersigned, hereby declare that to the best of my knowledge the clinical data presented in this report were compiled under my supervision, and accurately reflect the data obtained

W.A.A. Klöpping-Ketelaars, MD, PhD

Medical Investigator

28/12/04
Date / Signature

AUTHENTICATION BY THE CO-INVESTIGATORS

I, the undersigned, hereby declare that to the best of my knowledge the cholinesterase data presented in this report were compiled by me or under my supervision, and accurately reflect the data obtained.

H. Bekenes

Diagnostisch Centrum SSDZ

Co-Investigator cholinesterase assays

See next page
Date / Signature

I, the undersigned, hereby declare that to the best of my knowledge the urinary chemical analyses and dosing data presented in this report were compiled by me or under my supervision, and accurately reflect the data obtained.

H. Harper, PhD

Huntingdon Life Sciences Ltd.

Co-Investigator urinary and chemical analyses

See appendix 14.1.7.3. for signature
Date / Signature

6b/38

STATEMENT BY THE MEDICAL INVESTIGATOR

I, the undersigned, hereby declare that to the best of my knowledge the clinical data presented in this report were compiled under my supervision, and accurately reflect the data obtained

W.A.A. Klöpping-Ketelaars, MD, PhD

Medical Investigator

_____
Date / Signature

AUTHENTICATION BY THE CO-INVESTIGATORS

I, the undersigned, hereby declare that to the best of my knowledge the cholinesterase data presented in this report were compiled by me or under my supervision, and accurately reflect the data obtained.

28-12-04

H. Bekaneş

Diagnostisch Centrum SSDZ

Co-Investigator cholinesterase assays

_____
Date / Signature

I, the undersigned, hereby declare that to the best of my knowledge the urinary chemical analyses and dosing data presented in this report were compiled by me or under my supervision, and accurately reflect the data obtained.

H. Harper, PhD

Huntingdon Life Sciences Ltd.

Co-Investigator urinary and chemical analyses

See appendix 14.1.7.3. for signature

_____
Date / Signature

6b

Synopsis

Title of the trial:	Urinary excretion profile of Dimethoate and its metabolites after single oral administration of Dimethoate in male volunteers	
Investigators:	**Principal Investigator**: W.J.A Meuling, BSc **Medical Investigator**: W.A.A. Klöpping-Ketelaars, MD, PhD **Co-investigator**: H.Bekenes, Diagnostisch Centrum SSDZ, Delft, The Netherlands **Co-investigator**: H. Harper, PhD, Huntingdon Life Sciences Ltd. Eye, Suffolk, U.K.	
Study monitor	Scientific Consulting Company GmbH (SCC), Mikroforum Ring 1, D-55234 Wendelsheim. Germany. Phone +49 (0) 67349190, Fax +49 (0) 6734919191	
Study center:	TNO Nutrition and Food Research, Physiological Sciences Department, P.O. Box 360, NL-3700 AJ Zeist, the Netherlands. Phone.: +31-306944144, Fax: +31-306957224, Visitors address: Utrechtseweg 48, Zeist, the Netherlands	
Study period:	FSI 04AUG2003, LSO 07AUG2003	Clinical Phase: N.A.
Objectives:	Establishing in healthy male volunteers the urinary elimination profile of Dimethoate and its metabolites after a single oral administration of Dimethoate	
Methodology/Design:	The study was designed as an open, single dose study	
Number of subjects:	6 male subjects in age ranging from 19 – 32 years.	
Diagnosis and main criteria for inclusion:	Only healthy subjects according to in- and exclusion criteria, pre-study check-up results, physical examination. ECG recording, and pre-selection urinary metabolite profile and RBC and plasma cholinesterase activities, participated in this study	
Test product, dose, mode of administration, batch No:	A single oral administration of Dimethoate dissolved in demineralised water of about 0.03 mg/kg bw was given to all subjects on 04 August 2003.	
Reference therapy, dose, mode of administration, batch No:	Not applicable.	
Duration of treatment:	One single oral dose administered at once on 4th August, 2003. followed by blood, and urine sampling up to 72 h post dosing	
Criteria for evaluation:	Primarily: Urinary profiles of Dimethoate (DM), Omethoate (OM), Dimethoate carboxylic acid (DCA), Dimethyl dithiophosphate (DMDTP), and Dimethyl thiophosphate (DMTP). Safety: RBC-Che and plasma Che levels, ECG-recordings, heart rate and blood pressure	

Statistical methods:
Methods of (descriptive) statistics were used: arithmetic mean and standard deviations are calculated. Demographics and anthropometrics are reported descriptively and tabulated.

SUMMARY – CONCLUSIONS:

The present report describes the conduct and results of a study investigating the response to a single oral dose of Dimethoate (~0.03 mg/kg bw) in 6 healthy males on the urinary profiles of Dimethoate and selected metabolites. In this study approximately 0.03 mg/kg bw has been orally dosed per subject. In random urine samples of all subjects collected up to 72 hours post dosing, Dimethoate (DM), and selected metabolites as omethoate (OM). dimethoate carboxylic acid (DCA), dimethyl dithiophosphate (DMDTP) and dimethyl thiophosphate (DMTP) could be established. Based on calculation of total excreted amounts of all compounds, on average, 1.9 µg DM, 5.2 µg OM. 154 µg DMTP, 181 µg DMDTP and 837 µg DCA has been excreted. When these total amounts of quantified compounds (µmol) taken relative to the orally administered dose of Dimethoate (µmol), then the quantified compounds in urine accounted, on average. for 67.4 % ± 3.4; (range 63-73 %) of the administered dose. The following kinetic parameters were calculated: AUC, Cmax, and Tmax. From these results it was observed that after an oral dose of DM, urinary excretion of metabolites reaches a peak (Tmax) at about 2 hours post dosing for almost all compounds. with the exception of DMDTP and DMTP in subject 06 (6 h) and DMTP in subject 03 (6 h). Furthermore, is was observed that urinary excretion of almost all compounds is completed within 24-28 h with the exception of DMDTP and DMTP (48-60 h). Based on the obtained results, the determination of the selected compounds in urine is a suitable tool to measure the systemic absorbed dose e.g., in a field operator exposure study.

Efficacy results
Efficacy of Dimethoate has not been an objective in this study

Safety results
All participants completed the study and showed no signs or symptoms of adverse (systemic) effects due to or related to the oral administration of Dimethoate. Participants reported their well-being at the end of each study day. As was judged by the medical investigator, no significant clinically relevant laboratory pre-study, in-study and post-study check up results were reported. Neither inhibition of RBC-Che nor plasma-Che was observed in the study. Also safety ECG recordings, blood pressure and heart rate measurements in-study did not show clinically relevant changes or trends related to the treatment.

Conclusion
The following conclusions are drawn from this study:
- An oral administered dose of Dimethoate (~ 0,03 mg/kg bw) is well tolerated by male volunteers;
- Cholinesterase activities in RBC and plasma were not inhibited relative to subjects' average 95 %-confidence lower limit personal baseline values;
- No clinically relevant changes in ECG or trends in ECG recording were observed in the study;
- No clinically relevant heart rate, and blood pressure changes were established;
- In random urine samples of all subjects all 5 compounds could be established;
- The highest amount of metabolites excreted is (descending order), DCA>, DMDTP, DMTP>>, OM, DM;
- On average 67.4 % ± 3.4 of the oral dose is excreted as total amount of all compounds;
- The pharmacokinetic calculations showed that in most cases 2 hours post dosing excretion of compounds reached a peak and that excretion of most compounds is completed within 24-28 hours, with the exception of DMDTP and DMTP (40-60 h).

Date of report:
28nd December 2004

1 List of abbreviations (and definitions of terms)

ADI	: Acceptable Daily Intake
AE	: Adverse Event
ANOVA	: Analysis of Variance
ARfD	: Acute reference dose
BMI	: Body Mass Index
BP	: Blood Pressure
BW	: Body weight
CAS	: Chemical Abstract Services
Che	: Cholinesterase
CofA	: Certificate of Analysis
CRO	: Contract Research Organisation
CV	: Coefficient of Variation
DCA	: Dimethoate carboxylic acid (Dimethoate metabolite)
DM	: Dimethoate
DMDIP	: Dimethyl dithiophosphate (Dimethoate metabolite)
DTF	: Dimethoate Task Force
DMIP	: Dimethyl thiophosphate (Dimethoate metabolite)
ECG	: ElectroCardioGram
FSI	: First Subject Included in the study
GCP	: Good Clinical Practice
GLP	: Good Laboratory Practice
HLS	: Huntingdon Life Sciences Ltd. (Analytical laboratory)
HR	: Heart rate
ICD-10	: International Statistical Classification of Diseases and Related Health Problems
ICH	: International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
LC-MS	: Liquid Chromatography-Mass Spectrometry
LSO	: Last Subject Out of the study
METC	: Medisch Ethische ToetsingsCommissie (Medical Ethics Committee)
MSDS	: Material Safety and Data Sheet
NOAEL	: No Observed Adverse Effect Level
OECD	: Organisation for Economic Cooperation and Development
OM	: Omethoate (Dimethoate metabolite)
QAU	: Quality Assurance Unit
RBC	: Red Blood Cell
Retics	: Reticulocytes
SAE	: Serious Adverse Event
SCC	: Scientific Consulting Company
SOP	: Standard Operating Procedure
SSDZ	: Diagnostisch Centrum SSDZ; (Stichting Samenwerkende Delftse Ziekenhuizen)
TNO	: Nederlandse organisatie voor Toegepast Natuurwetenschappelijk Onderzoek (Netherlands Organization for Applied Scientific Research)
TSDS	: Technical Safety and Data Sheet
WBC	: White Blood Cell
WHO	: World health Organisation
WMO	: Wet medisch onderzoek met mensen (Law 'medical research with human subjects')

2 Ethics

2.1 Independent Ethics Committee

The study protocol has been drafted in accordance with the current ICH Guideline for Good Clinical Practice (ICH Topic E6, Guideline for Good Clinical Practice, adopted 01-05-1996 and implemented 17-01-1997).
The protocol was submitted to the Medical Ethics Committee (METC-TNO) and approval had been given on 14 March 2003

2.2 Ethical conduct of the study

The study was conducted according to:

1. The current revision (52nd) of the World Medical Association General Assembly, Declaration of Helsinki (Edinburgh, Scotland, October 2000), and the Note of clarification on paragraph 29 added at the WMA General Assembly, Washington, USA, October 2002;
2. The ICH Guidelines for Good Clinical Practice (ICH Topic E6, adopted 01-05-1996 and implemented 17-01-1997);
3. The Dutch Medical Research involving human Subjects Act („Wet medisch-wetenschappelijk onderzoek met mensen", WMO, 01-12-1999);
4. The current national regulations.

2.3 Subject information and consent

Nineteen positively responding potential candidates (first recruitment) and five positively responding potential candidates (second recruitment) were invited to come to TNO for an oral briefing in the presence of several members of the project team during which they were informed about the aim, the procedures, the constraints, the insurance cover and the financial compensation of the study. The oral briefings were held on 02, 10 and 22 July 2003, respectively. Seven positively responding and invited potential candidates did not show up at the oral briefings without any notice.
Prior to the meeting, all potential candidates received a copy of the information package 'Schriftelijke informatie proefpersonen' (P4802 B01; Appendix 14 1.1 also including English translation), that fully covered the information that was actually given verbally during the meeting. After the respondents became familiar with the content and procedures of the study, those who were interested to participate undersigned, in duplicate, the informed consent form (P4802 F01 in Dutch; appendix 14 1 4), one of which they retained. Pre-screening: One subject was excluded due to a positive drug screen and three subjects based on either out of range clinical chemistry results or inclusion criteria. Pre-selection was completed by a total group of 14 candidates (9+5extra). Finally, based on urinary metabolite profiles and cholinesterase (Che) activity levels 8 subjects were eligible for the study, of which 2 subjects were assigned substitutes. This decision was made by the medical investigator and was agreed by the sponsor.

3 Investigators and study administrative structure

This study was conducted under the supervision and responsibility of the Principal Investigator. He was responsible for the actual oral administration and the overall conduct of the study. He was responsible for the dispatch to the chemical laboratory (Huntingdon Life Sciences Ltd.) of the dosing solution and frozen urine samples and for the dispatch of hemolyzed blood samples to the clinical chemistry laboratory Stichting Samenwerkende Delftse Ziekenhuizen (SSDZ). He was also responsible for the organisation of the study, drafting the study protocol, collecting of the data and drafting the study report.

The sponsor was responsible for the analytical determination of Dimethoate, and the selected metabolites omethoate, dimethoate carboxylic acid (DCA), dimethyl dithiophosphate (DMDTP), and dimethyl thiophosphate (DMTP) in urine and Dimethoate in the dosing solution according to validated analytical procedures by a GLP certified laboratory (Huntingdon Life Sciences Ltd) and for the prompt forwarding of audited analysis results to TNO for inclusion in the audited final report.

The Medical Investigator was directly responsible for the selection, documentation, interpretation and reporting of Adverse Events (AEs) and Serious Adverse Events (SAEs) and the general safety of the subjects. Parts of the medical aspects of the study (e.g., screening, eligibility, interpretation of ECG's, registration of AEs, were delegated to an assistant medical investigator appointed by the management.

The Co-investigator was responsible for the enzymatic determination of plasma and RBC cholinesterase in hemolysed blood samples. Qualified personnel appointed by the management carried out the actual enzymatic assays.

Chemical analysis of Dimethoate and metabolites in urine samples and dosing solution was carried out by a GLP certified laboratory (Huntingdon Life Sciences Ltd, Eye, Suffolk, UK).

Testing facilities

The study has been conducted by:
TNO Nutrition and Food Research
Physiological Sciences Department
Utrechtseweg 48
P.O. Box 360
3700 AJ ZEIST
Phone : +31 30 69 44 14 4
Fax : +31 30 69 57 22 4

Contributors

The following persons of TNO were actually involved in this study:

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Medical investigator
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S Sukhraj
J Jansen
A E.A M. Speulman-Saat

Facility for the cholinesterase analysis
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Department of Clinical Chemistry
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Responsible person at SSDZ
TeamLeader Chemistry Unit
H Bekenes
Phone : +31 15 260 45 23
Fax: : +31 15 256 81 03
Email : Bekenes@RDGG.nl

Facility for chemical analysis (urine and dosing solution)
Analysis of urine metabolites and dosing solutions has been carried out by the following selected GLP certified laboratory.

Huntingdon Life Sciences Ltd,
Eye
Suffolk, IP23 7PX
United Kingdom

Responsible person at Huntingdon Life Sciences Ltd.
Residue Analysis, ERC
H. Harper, PhD
Phone : +44 1379 67 21 72
Fax: : +44 1379 67 21 00
Email : harper@ukorg.huntingdon.com

Retention of samples and records

The following documents will be retained in the archives of INO Nutrition and Food Research during 15 years after the final report has been issued.

1. Master copies of the approved study protocol, and final report
2. All documents containing personal data of individual trial subjects
3. Raw data (source documents or authenticated copies of these) of analyses conducted at INO Nutrition and Food Research and at the Diagnostisch Centrum, SSDZ, and the final audited analytical report of Huntingdon Life Sciences Ltd.
4. Correspondence
5. All other information related to tests and analyses conducted at INO Nutrition and Food Research

The following samples and specimens will be retained in appropriate facilities of INO Nutrition and Food Research:

1. A representative part of the study substance which will be retained for 5 years
2. The remainder of the in-study urine samples which will be retained for three months after the final report has been approved by the sponsor. After expiration of the above indicated storage time period the sponsor will be consulted and will in writing indicate the future of the stored material.

All remaining samples will be discarded after this period at the investigators site.

However, under no circumstances will any data be discarded without the sponsor's consent

4 Introduction

In agriculture, workers may be exposed either dermally, inhalatory and/or orally to Dimethoate by performing different tasks. This study is undertaken to investigate the urinary excretion profile of Dimethoate and selected metabolites in humans after a single oral administration of Dimethoate. The results of this study could be used to investigate the exposure of agricultural workers (operators) to Dimethoate in typical field exposure scenarios.

The study protocol has been drafted in accordance with, and has been conducted according to the ICH Guideline for Good Clinical Practice (ICH Topic E6, Guideline for Good Clinical Practice, adopted 01-05-1996 and implemented 17-01-1997).

5 Study objective

The objective of the present study is to establish in healthy male volunteers the urinary elimination profile of Dimethoate and selected metabolites after a single oral administration of Dimethoate.

The study can be considered as a pre-study for a field exposure study to investigate the systemically available dose of operators under typical use conditions

6 Investigational plan

The investigational plan has been outlined in detail in Protocol P4802, dated 12 May 2003, version revised final 1 (see Appendix 14.1.1). The protocol will be mentioned in this report further as 'Protocol P4802'.

6.1 Overall study design and plan description

The study has been designed as an open, single dose study. The investigational plan has been outlined in detail in Protocol P4802 (see Appendix 14.1.1)

6.2 Discussion of study design and choice of control group

In this study no control group has been used.

6.3 Selection of study population

The subjects in this study were recruited from the pool of volunteers of TNO Nutrition and Food Research in compliance with the following in- and exclusion criteria outlined in detail in Protocol P4802 (Appendix 14.1.1).

Inclusion criteria:

1. Age ≥18 years ≤ 45 years on Day 01
2. BMI (Body Mass Index) ≥ 22 - ≤ 28 kg/m^2
3. Healthy as assessed by health questionnaire, physical examination and clinical chemistry (fasting state) including: haematology (RBC, WBC, platelets, Ht, Hb, Retics) and WBC blood differentiation; Clinical chemistry profile (ALT, ALP, ASI, γ-GT, albumine, total bilirubin, creatinin, glucose and urea); Drug screen in urine (methadon, benzodiapines, cocaine, amphetamine/methamphetamine, opiates, tetrahydrocannabiol, barbiturates, tricyclic antidepressiva); Dipstick urinalysis (protein, glucose, leucocytes, erythrocytes, nitrite, pH, ketones, urobilinogen, bilirubin); if the dipstick test would have given values above the normal range for leucocytes, blood or protein, a microscopic inspection of urine sediment was done.
4. Having given their written informed consent
5. Willing to comply with the study procedures
6. Voluntary participation
7. Willing to refrain from blood donation 30 days in advance of Day 01 and during the whole study
8. Willing to accept use of all anonymized data, including publication, and the confidential use and storage of all data
9. Willing to accept the disclosure of the financial benefit of participation in the study to the authorities concerned
10. Willing to refrain from intake of nutritional supplements during a period of 2 weeks prior to the study and ending at 72 h after the oral administration in the study

Exclusion criteria
Subjects with one or more of the following characteristics were excluded from participation:

1. Participation in any clinical trial including blood sampling and/or administration of substances up to 90 days before Day 01 of this study
2. Under medical treatment of a specialist in the past and present for a reason that may affect the study outcome
3. Use of prescribed medication (paracetamol excluded)
4. Alcohol consumption more than 28 units/week; (1 unit of alcohol equals 10 grams of ethanol)
5. Occupational exposure to, contact with, or use of agrochemicals
6. Physical training exceeding 10 hours of heavy weekly training or planning to train for an event requiring extreme physical training like triathlon or marathon
7. High background levels of the urinary metabolites to be measured, in two blank urine samples collected within three weeks prior to Day 01 of the study
8. Large variation (C.V. >15 %) of plasma and RBC cholinesterase activities of three blank samples collected within three weeks prior to Day 01 of the study
9. TNO personnel and their relatives in the first and second remove
10. Having unsuitable veins for blood sampling
11. Not having a general practitioner
12. Not willing to accept information-transfer concerning participation in the study concerning participation in the study, or information regarding his health, like laboratory results, findings at anamnesis or physical examination and eventual adverse events to and from his general practitioner or his objection concerning participation
13. Not willing to accept transfer of medical information to and from the subjects' general practitioner when results of the study urgently demand for it, like laboratory results, findings at anamnesis or physical examination and eventual adverse events

6.4 Treatment

6.4.1 *Oral administration*
To each subject a single dose of Dimethoate in demineralised water (25 mL) has been administered at a dose level of about 0.03 mg/kg bw.

6.4.2 *Identity of investigational product*

- Common name : Dimethoate
- Chemical name : O,O-Dimethyl S-methylcarbamoylmethyl phosphorodithioate
- CAS registry number : 60-51-5
- Recieved : 15 april 2004
- Batch number : 20522-00
- Aim : insecticide
- Molecular formula : $C_5H_{12}NO_3PS_2$
- Molecular weight : 229.3
- Galenic form : white powder
- Water solubility : 39.8 g/L (20°C)
- Storage conditions : refrigerated (+4°-10°C) in the dark
- Expiry date : 30-04-2008
- TNO reference No. : 30095

Relevant toxicological properties
ARfD (Acute reference dose) : 0.03 mg/kg bw

MSDS and TSDS of test substance
A Material Safety and Data Sheet of the manufacturer of Dimethoate and a Test Substance Data Sheet received from the monitor (SCC) are given in Appendix 14.1.2.

All documents with regard to the test substance were finally archived at TNO Nutrition and Food Research.

6.4.3 *Pre-selection tests*
Urine: To investigate whether subjects urine comprises possible substances that may affect the analysis of the expected metabolites, two blank urine samples collected over 2 times 24 hours were collected within three weeks prior to the actual administration (Day 01). These samples were sent to Huntingdon Ltd and analysed. In case of a positive result, subjects were excluded from participation
Cholinesterase: Also the individual baseline levels of plasma and RBC cholinesterase activities were established. Therefore, three blank blood samples were collected within three weeks prior to Day 01 of the study. From the analysis results the individual mean and 95 %-confidence limits were calculated. In case a large variation (C.V. >15 %) in activities occurs, subjects were excluded from participation.

6.4.4 *Method of assigning subjects to treatment*
Subjects were allocated to an entry number in order of arrival on Day 01 of the study. No assigning of entry numbers to treatment took place, since only one treatment (oral) was scheduled in this study.

6.4.5 *Selection of doses in the study*
The selection of the oral dose has been based on toxicological results in reported animal and human investigations. From these results a so-called acute reference dose (ArfD) has been deduced. Further, a no effect level of 0.3 mg/kg bw was deduced from a human study. Using an assessment factor of 10 for interindividual variations, and the relevant NOEL of 0.3 mg/kg bw, the ARfD is calculated to be: ARfD = 0.3 mg/kg bw/day : 10 = 0.03 mg/kg bw/day.

6.4.6 *Selection and timing of dose for each subject*
All subjects arrived in the fasting state at TNO on Day 01 of the study. After a blank blood sample was drawn and an urine sample has been provided, a standardized breakfast (2 buns) was served with tea or coffee. Thereafter, subjects took their oral dose (t=0) of Dimethoate staggered in about ten minutes intervals on Day 01 from 09:40 till 10:30 h of the study.

6.4.7 *Blinding*
Since this study was designed as an open, single oral dose study blinding/unblinding was not applicable.

6.4.8 *Prior and concomitant therapy / treatment*
At each visit to TNO subjects intake of medication, as well as prescribed medication or so-called over-the counter drugs were recorded (Well-being questionnaire).

6.4.9 *Treatment compliance measurement*
The single oral dose in each subject was administered by the principal investigator at the respective study Day. No other treatment compliance check took place thereafter.

6.5 Efficacy and safety variables

6.5.1 *Efficacy and safety measurements assessed*
No efficacy measurements were carried out in this study.
Pre-study and in-study RBC and Plasma cholinesterase measurements and recordings of ECG were performed at pre-determined time intervals. ECG recordings were established five times during the clinical part of the study.
Recording scheme: pre-screening, pre-dose, and at about 3 h, 24 h and 72 h post dosing.
Blood pressure (BP) and heart rate (HR) were established: pre-screening, pre-dose, 3 h, 5 h and 24 h post-dosing
Body weights were established pre-screening, and prior to oral dosing. The cholinesterase results (in-study) were compared relative to the personal baseline levels (using Excel spreadsheets) by the principal investigator while the ECG results were examined by an expert physician (cardiologist). Raw data are filed and archived.

6.5.2 *Appropriateness of measurements*
The cholinesterase (RBC and plasma) measurements used in this study are generally agreed routine measurements for evaluation of cholinesterase inhibition by e.g., organophosphates and carbamates. For details see Appendix 14.1.1.; 8.9). Personal 95 % confidence limits of cholinesterase activities could be calculated as at least four (unexposed) baseline levels were established. These values were used to compare the achieved cholinesterase activity in-study levels (after treatment), and to conclude whether inhibition was present.
The ECG recordings pre-study, in-study (up to 72 h) were used for evaluation of the health status due to dosing with Dimethoate.

6.5.3 *Primary efficacy variable(s)*
No efficacy variables were established in this study.

6.5.4 *Drug concentration measurements*
Two aliquots (~2 mL) of the oral dosing solution (stock) and two aliquots (~2 mL) of a prepared diluted sample (500 µL in 25 mL demineralised water) were transferred in pre-labelled sample tubes and stored in a freezer at ≤ -18°C until transportation. These samples were sent on dry ice by courier to the analytical chemical laboratory (Huntingdon Life Sciences Ltd.) to establish the Dimethoate concentration.
The development and validation of the analytical methods (parent compound and metabolite measurement in urine and dosing solution) have been conducted by the chosen analytical laboratory.

6.6 Data quality assurance

TNO Nutrition and Food Research has been inspected by the Public Health Inspectorate for compliance with the principles of Good Clinical Practice for the conduct of clinical trials according to the EC directive 92/507/EC and the ICH Guidelines for Good Clinical Practice (CPMC)/ICH/135/95). A written statement is supplied in P4802 B06, (see Appendix 14.1.1)

The cholinesterase analyses were performed by Diagnostisch Centre SSDZ, which were audited by the QAU (see for the TNO audit certificate Appendix 14.1.6.), and therefore are included in the GCP statement. All non-clinical phases of the study that were conducted at TNO Nutrition and Food Research were carried out to the internationally accepted standards of Good Laboratory Practice. The analysis in urine was performed according to GLP by a

GLP certified laboratory. The respective documents including an QA statement are part of the analytical report (see Appendix 14.1 7.3).

6.7 Kinetics

6.7.1 *Preparation of dosing solution*

Preparation of the study treatment solution (stock):

About 48 mg Dimethoate was weighed and dissolved in 12 mL demineralised water by gentle swirling. This stock solution has been used for the individual oral administrations and contained, based on weighing, 3.969 mg Dimethoate per mL solution.

The preparation (weighing, dissolution) of this stock solution has been carried out by the principal investigator on Day 01 of the study just prior to the first administration and has been checked and confirmed (weighing and dissolution) by the medical investigator. Raw data has been filed and archived.

6.7.2 *Oral dosing*

Based on the individual body weights of the subjects recorded prior to administration, from the aforementioned stock solution per subject a range of 510-670 µL has been added to a glass cup containing about 25 mL demineralised water and gently swirled. This solution has been administered orally at t=0 h. The glass cup was washed two times with about 25 mL demineralised water, gently swirled and also orally administered. Two aliquots (~2 mL) of the stock solution has been decanted in pre-labelled sample tubes directly after the last administration and stored in a freezer at ≤ -18°C awaiting transportation and analytical determination of the Dimethoate concentration. In addition a diluted sample (500 µL in 25 mL) has been prepared and also two aliquots (~2 mL) of the homogenous solution directly after the last administration were transferred in pre-labelled sample tubes and stored in a freezer at ≤ -18°C awaiting transportation for analytical determination of the Dimethoate concentration. Subjects body weights, the actual volume pipetted, and administration time, were recorded and registered (Excel spreadsheet) by the principal investigator and archived (raw data) at TNO Nutrition and Food Research. Preparation and administration of the oral dosing has been carried out by the principal investigator within a time span of 2 hours.

6.7.3 *Urine sample collection*

All produced urine (in-study) has been collected fractionated in containers from the onset of the oral administration up to about 72 hours post-dosing, according to the pre-determined sampling scheme (see below). To establish baseline values prior to the start of the oral administration subjects were instructed to empty their bladder completely and collect the urine sample (blank of Day 01). Subjects received information how to collect samples and were instructed to record the time of voiding onto the label of the particular container and to store the container at home in a cool and dark place before transportation to TNO. Upon arrival at TNO recorded voiding times were registered and the amount of urine was determined by weighing and registered on forms. Two aliquots of 50-80 mL out of each urine fraction were taken and transferred to a prelabelled container and about 1 mL to pre-labelled tubes, the remainder was discarded. All sub-samples were stored in a freezer at ≤ -18°C awaiting transportation either to Huntingdon Ltd. or the clinical chemistry department of TNO (creatinine content).

Transportation to Huntingdon Ltd. has been done on dry ice by courier.

Sampling scheme: overnight (blank of Day 01), 0-4, 4-8, 8-12, 12-24 and 24-36, 36-48h and 48-72h after the oral administration (8 samples per subject; totally 48 samples).

6.7.4 *Blood sample collection*

Blood sampling (in-study) has been performed by finger-tip puncture using lancets pre-dose, and at pre-determined time intervals in the study. All samples were collected into heparinised calibrated capillaries (40 µL). After sampling, the capillary has been placed in a prelabelled tube containing 1 mL of a saponin solution (1 mg/mL). The tubes were capped and shaken to hemolyse the blood. The hemolysates were stored in a refrigerator at +4°C – 10°C awaiting transportation to, and determination of cholinesterase activities by SSDZ the same day. After the start of the oral administration blood sampling were performed up to about 72 hours post dosing according to the following sampling scheme.
Sampling scheme (hemolysates): pre-dose, 4 h, 24 h and 72 h after the oral administration (4 samples per subject; totally 24 samples)
Transportation to SSDZ has been done by courier on melted ice, the same day of collection.

6.8 Chemical analysis of urine samples

The analyses of Dimethoate, omethoate and its urinary metabolites, but also Dimethoate in the dosing and diluted dosing solution has been carried out by Huntingdon Life Sciences Ltd. All urine samples collected in the pre-selection and in-study have been analysed for Dimethoate (DM), omethoate (OM), DCA, DMDTP, and DMTP according to validated LC-MS methods. A detailed report including a GLP statement is given in appendix 14.1.7.3.
In all urine (in-study) samples the creatinine content of urine have been analyzed according to a standardised enzymatic method by the clinical chemistry department of TNO Nutrition and Food Research

6.9 Cholinesterase assay in hemolyzed blood

All hemolysed blood samples were analysed enzymatically for plasma and RBC cholinesterase according to a validated method (modified Ellman method) described by Meuling et al. 1992 [2]. Both plasma and RBC cholinesterase activities present in the hemolysates were determined with a modified colorimetric Ellman method using specific substrates (plasma; s-butyrylthiocholine and RBC; acetyl(β-methyl)-thiocholine). In each analysis run quality control samples containing both known cholinesterase activities were co-analyzed.

6.10 Statistical methods planned in the protocol and determination of sample size

6.10.1 *Statistical and analytical plans*

No statistical methods has been used to evaluate the data. Arithmetic means and standard deviations of the study parameters were calculated using Excel spreadsheets. Calculation were carried out using Excel spreadsheets, therefore calculations carried out using the tabulated data may lead to minor differences due to rounding-off procedures
Anthropometric and demographic data of the subjects are presented descriptively and tabulated.

6.10.2 *Determination of sample size*

The motivation of the number of 6 subjects for the treatment was based on experience and on the minimal number of subjects needed to calculate arithmetic means and standard deviations including (limited) insight in the inter-individual variation. The strategy of sampling and the number of samples was selected to provide information on the urinary excretion profile of Dimethoate and selected metabolites and the possible

effect on plasma and RBC cholinesterase activities, following a single oral administration of Dimethoate.

6.11 Changes in the conduct of the study or planned analyses

Since not all candidates that positively responded to the invitation came to the oral information session (2 and 10 July 2003), it was decided after discussion with the sponsor to arrange a second recruitment by sending out letters (n=180) to possible candidates from the TNO Nutrition and Food Research subjects database to ascertain a sufficient number of volunteers for the main study. Five positive respondents were invited to come to TNO in the fasting state for an extra oral information session (22 July 2003). After giving their written informed consent all candidates were subjected to a pre-screening conform the protocol (see appendix 14.1.1), and started immediately with the pre-selection phase, i.e , urine collection and blood sampling for cholinesterase measurements. Collected urine samples were sent on 28 July 2003 by courier on dry ice to Huntingdon Ltd. Collected hemolysates were sent by courier to SSDZ on melted ice, the day of collection and analysed.

7 Study subjects

7.1 Disposition of subjects

Six (+2 substitutes) healthy males participated in the study. Two healthy males were assigned as first (actually present on day 01) and second substitute (on call on day 01) in the study. The first subject (#01) (FSI) included in the study, entered the study on Day 01, dated 4th, August. The last subject (#06) out of the study (LSO) left TNO Nutrition and Food Research on Day 07th, August. Since all subjects arrived on time on Day 01, it was not necessary to include either the first or the second substitute in the study. No subject left the study prematurely.

7.2 Protocol deviations

The study was conducted following the mutually agreed and approved study protocol P4802 [1]. Deviations from this protocol are mentioned hereafter.

- Instead of one, two recruitment phases and two oral information meetings were held to ascertain a sufficient number of volunteers for the main study.
- Demineralised water (Millipore®) was used for the Dimethoate preparation of the oral dosing solution instead of distilled water.
- In the protocol the following metabolites are mentioned: DIP (dithiophosphate) and IP (thiophosphate), this should read DMDTP (dimethyl dithiophosphate) and DMIP (dimethyl thiophosphate).

7.3 Blind breakage

Since the study was designed as an open study, blind breakage was not applicable.

8 Results

8.1 Demographic and other baseline characteristics

Table 1. Demographic and other baseline characteristics of subjects (n=6) at inclusion

Gender (n=6)	Mean ± SD	Range
Age (years)	25.0 ± 5.2	19 – 32
Body weight (kg)	76.5 ± 8.1	65.9 – 88.4
Height (m)	1.82 ± 0.07	1.75 – 1.94
BMI (kg/m^2)*	23.0 ± 1.4	21.5 - 23.5

8.2 Measurement of treatment compliance

On all scheduled visits at TNO, all subjects were asked whether all produced urine had been collected in completeness which was confirmed by all subjects.

8.3 Efficacy results

Efficacy has been not been an objective of this study.

* BMI= Body Mass Index: is the ratio between the body weight (kg) and the square of the height in meters of a person and is a (healthy) weight index

9 Kinetic results

This study has been conducted besides protocol deviations mentioned in paragraph 7.2, exactly as described in Protocol P4802. In the following paragraphs the main findings are summarised. The detailed findings are provided in appendices 14.1.7.1 – 14.1.7.3 as well as 14.2.1 – 14.2.4.

9.1 Dimethoate concentration in dosing solution

The concentration of Dimethoate in the dosing solution (4802-D01 A and B) and in the diluted dosing solution (4802-D02 A and B) were established by the chemical laboratory Based on weighing the calculated concentration of the stock dosing solution amounted to 3969 µg/mL (Appendix 14.2.4)

Table 2: Analysed concentrations of Dimethoate in the dosing solution

Sample number HLS	Sample number TNO	Dimethoate concentration (µg/mL)
03/SCI/2789	4802-D01-A	3830
03/SCI/2790	4802-D01-B	3400
	On average	**3615 ±304**
03/SCI/2791	4801-D02-A	74.5
03/SCI/2792	4801-D02-B	74.7
	On average	**74.6 ±0.14**

The analytical results of the dosing (stock) solution are lower than the calculated concentration based on weighing; 3615 µg/mL versus 3969 µg/mL. For the diluted stock solution (500 µL added to 25 mL = 51 times) the analytical results amounts on average to 74.6 x 51= 3804.6 µg/mL, which is closer to the calculated concentration based on weighted substance (see Appendix 14.1.7.2 and 14.1.7.3) for the analytical results.

Note: 1) In all calculations further provided in this report, the analytically verified concentration of 3615 µg/mL has been used as the average stock solution concentration.
2) All calculations have been carried out using raw data and Excel spreadsheets therefore re-calculation carried out otherwise may result in slightly different figures (rounding-off).

9.2 Oral dosing

It was the intention that all subjects were dosed with Dimethoate approximately to a level of 0.03 mg/kg bw. Therefore, prior to dosing all body weights were measured and recorded. Based on the nominal Dimethoate concentration of the stock solution (3.969 mg/mL) and the body weights of the subject a volume of 510-670 µL taken out the stock solution was added to 25 mL of demineralised water and gently swirreled before oral administration took place. Subjects were dosed with 10 minutes intervals. The following calculated doses were administered

Table 3. Administered oral dose (mg/kg bw) per subject

Subject #	Oral dose of Dimethoate (mg/kg bw)*
01	0.0277
02	0.0276
03	0.0276
04	0.0274
05	0.0276
06	0.0276

* based on analytically verified concentration (See also Appendix 14.2.4)

9.3 Pre-selection RBC-Che and plasma-Che results

To determine the biological variation of RBC and plasma cholinesterase activity, three control blood samples (finger prick) were taken of all subjects within seven days prior to Day 01 of the study. These samples were hemolysed and stored refrigerated at +4 °C-10 °C until transportation to SSDZ on melted ice for analysis at the same day. The calculated results (mean value and coefficient of variation) show that all individual measurements of all subjects (n=14) were well within the set inclusion criteria (CV < 15 %) for each subject.

RBC-Che : mean Coef. Var.: 6.53 % (range : 0.68- 12.9 %) (n=14)
Plasma-Che : mean Coef. Var.: 3.02 % (range: 0.46 - 7.44 %) (n=14)

The co-analysed control sera and one pooled sample (#5) proofed the reliability of the sample results.
The individual results of all 6 in-study subjects are summarised in tables 4a and b.

Table 4a. Individual pre-selection plasma cholinesterase results (mean ± s.d)

Subject Entry number	Corresponding subject pre-entry number	Plasma Che-results (U/L) Mean ± s.d.	Coef. of Variation (%) (n=3)
01	111	8643 ± 39.4	0.46
02	118	5937 ± 441.6	7.44
03	103	7692 ± 346.6	4.51
04	107	6102 ± 272.6	4.47
05	108	6304 ± 259.3	4.11
06	102	6306 ± 411.5	6.53

Table 4b. Individual pre-selection RBC-cholinesterase results (mean ± s.d)

Subject Entry number	Corresponding subject pre-entry number	RBC-Che results (U/L) Mean ± s.d.	Coef. of Variation (%) (n=3)
01	111	7155 ± 313.4	4.38
02	118	6696 ± 326.2	4.87
03	103	7803 ± 757.3	9.71
04	107	7407 ± 420.4	5.68
05	108	7871 ± 842.1	10.70
06	102	7671 ± 989.2	12.90

9.4 In-study RBC-Che and plasma-Che results

RBC and plasma cholinesterase has been assayed in hemolysed blood samples in-study according to the following sampling scheme: Pre-dose, 4 h, 24 h and 72 h post dosing

All samples were treated as described in paragraph 9.3 and finally analysed. The co-analysed control sera and one pool sample (#5) proofed the reliability of the analytical results of the samples.

For the individual data the reader is also referred to paragraph 14.2.2.

Plasma-Che activity levels of the subjects are summarised in tables 5a and 5b. RBC-Che activity levels of the subjects are summarised in tables 6a and 6b. The post-dose plasma-Che and RBC-Che acitivity levels showed a low coefficient of variation, which was as follows:

Plasma-Che : mean Coef. Var.: 2.70 % (range : 0.75 - 5.32 %) (n=6)
RBC-Che : mean Coef. Var.: 2.75 % (range : 0.40 - 5.31 %) (n=6)
See also appendix 14.2.2. for all individual in-study results.

This variation was lower than the observed variation in the pre-selection samples.

Pre-dose Che values in plasma and RBC and the respective pre-selection figures for each individual were taken together to establish the non-treated 'baseline values' as well as corresponding 95 %-confidence limits as presented in tables 5a and 6a, respectively. In table 5b plasma-Che levels after Dimethoate administration are shown to compare with plasma-Che levels before dosing as presented in table 5a. In table 6b RBC-Che levels after Dimethoate administration are shown to compare with RBC-Che levels before dosing as presented in table 6a.

Table 5a. Individual selection and pre-dose plasma-Che activity levels (mean ± s.d and 95 %-confidence limits)

Subject Entry number	Pre-selection baseline value Mean ± s.d.	Pre-dose value (t=0 h)	Plasma-Che results (U/L) (including t=0 h) Mean ± s.d.	Baseline 95 %-Confidence limits lower upper
01	8643 ± 39.4	8045	8493 ± 300.6	7892 – 9094
02	5937 ± 441.6	5811	5906 ± 366.0	5174 – 6638
03	7692 ± 346.6	7713	7697 ± 283.2	7131- 8263
04	6102 ± 272.6	6067	6093 ± 223.3	5646 – 6540
05	6304 ± 259.3	6018	6233 ± 255.6	5722 – 6744
06	6306 ± 411.5	6685	6401 ± 385.8	5629 – 7172

Table 5b. In-study (post-dose) individual plasma-Che activity levels

Subject Entry number	In-study Plasma-Che results (U/L)			Plasma-Che results (U/L) Mean ± s.d.	Baseline 95 %-Confidence limits (=table 5a) lower upper
	t = 4 h	t = 24 h	t = 72 h		
01	8213	8623	8025	8287 ± 305.8	7892 – 9094
02	5884	5822	5799	5835 ± 44.0	5174 – 6638
03	8011	8206	7737	7985 ± 235.6	7131 – 8263
04	6163	6456	6207	6275 ± 158.0	5646 – 6540
05	5980	6092	6055	6042 ± 57.1	5722– 6744
06	6390	7106	6715	6737 ± 358.5	5629 – 7172

Table 6a. Individual selection and pre-dose RBC-Che activity levels (mean ± s.d and 95 %-confidence limits)

Subject Entry number	Pre-selection baseline value Mean ± s.d.	Pre-dose value (t=0 h)	RBC-Che results (U/l) (including t=0 h) Mean ± s.d.	Baseline 95 %-Confidence limits lower upper
01	7155 ± 313.4	6467	6983 ± 428.7	6126 – 7840
02	6696 ± 326.2	6772	6715 ± 269.0	6177 – 7253
03	7803 ± 757.3	7225	7659 ± 682.6	6293 – 9024
04	7407 ± 420.4	7101	7331 ± 375.9	6579 – 8083
05	7871 ± 842.1	6960	7643 ± 824.8	5994 – 9293
06	7671 ± 989.2	7094	7527 ± 857.6	5811 – 9242

Table 6b. In-study (post-dose) individual RBC-Che activity levels

Subject Entry number	In-study RBC-Che results (U/L)			RBC-Che results (U/L) Mean ± s.d.	Baseline 95 %-Confidence limits (=table 6a) lower upper
	t = 4 h	t = 24 h	t = 72 h		
01	6782	6996	6434	6737 ± 283.7	6126 – 7840
02	6762	6955	6751	6823 ± 114.7	6177 – 7253
03	7900	7617	7109	7542 ± 400.8	6293 – 9024
04	6988	7236	7084	7103 ± 125.0	6579 – 8083
05	6935	6987	6977	6966 ± 27.6	5994 – 9293
06	7791	7426	7579	7599 ± 183.3	5811 – 9242

Comparing tables 5a and 5b and tables 6a and 6b, practically no differences are noted between the in-study (post-treatment) Che levels and the mean pre-dose Che-levels for any subject. All post-treatment Che-activities including the 4 hours figures as the potential time of peak effect, are well within the 95 %-confidence limits of the Che-levels for the untreated 'baseline' period.

9.5 Urinary Dimethoate and metabolites results

All collected in-study urine samples (48) were sent to Huntingdon Life Science Ltd. for analysis of Dimethoate (DM), omethoate (OM), DCA, DMDTP and DMTP. Analysis showed that in various urine samples of all subjects collected up to 72 hours post dosing, Dimethoate (DM), omethoate (OM), DCA, DMDTP and DMTP could be measured. Table 7 summarises the oral administered dose, the urinary excreted amounts of the various

metabolites and the total excreted amounts in mg and μmol and taken relative to the administered dose (%) on a mol/mol basis (in brackets) per subject.

Table 7. Oral administered dose and excreted amount of metabolite (single and totally) per subject

Subject #	Oral administered dose (mg) [μmol]	Amount of metabolite excreted (mg) [Percentage of administered dose on mol/mol basis]					Total of metabolites excreted (mg)	Total of metabolites excreted (μmol)
		Dimethoate	Omethoate	DCA	DMDTP	DMTP		
01	2.10 [9.15]	0.0013 [0.06]	0.0121 [0.62]	0.6570 [33.22]	0.1496 [10.40]	0.2935 [22.74]	1.1134	6.1323
02	2.13 [9.30]	0.0009 [0.04]	0.0023 [0.12]	0.8549 [42.50]	0.2645 [18.09]	0.1157 [8.82]	1.2383	6.4719
03	2.42 [10.57]	0.0032 [0.13]	0.0067 [0.30]	0.8711 [38.13]	0.1756 [10.57]	0.2016 [13.52]	1.2582	6.6204
04	1.84 [8.04]	0.0020 [0.11]	0.0027 [0.16]	0.8782 [50.51]	0.1199 [9.49]	0.0657 [5.79]	1.0685	5.3117
05	1.88 [8.20]	0.0010 [0.06]	0.0013 [0.07]	0.7420 [41.85]	0.2363 [18.33]	0.0719 [6.21]	1.0525	5.4553
06	2.17 [9.46]	0.0033 [0.15]	0.0058 [0.29]	1.0156 [49.65]	0.1392 [9.36]	0.1755 [13.15]	1.3394	6.8683
Mean ± s.d.	2.09 ± 0.211 [9.12 ± 0.92]	0.0019 ± 0.0010 [0.092 ± 0.044]	0.0052 ± 0.0040 [0.259 ± 0.200]	0.8365 ± 0.1237 [42.64 ± 6.64]	0.1809 ± 0.0575 [12.71 ± 4.29]	0.1540 ± 0.0874 [11.70 ± 6.33]	1.1784 ± 0.1166	6.1433 ± 0.6366

Table 8 summarises the average ± s.d., as well as the range of excreted amount of the various compounds observed for the 6 volunteers.

Table 8. Mean ± sd and range of the various excreted amounts of the urinary compounds (mg and μmol) and expressed relative to the administered dose (%)

Compound	Mean ± s.d. (mg)	Range (mg)	Mean ± s.d. (μmol)	% excreted of oral dose
Dimethoate (DM)	0.0019 ± 0.0010	0.0009-0.0033	0.0083 ± 0.0044	0.092
Omethoate (OM)	0.0052 ± 0.0040	0.0013-0.0121	0.0244 ± 0.0188	0.259
DCA	0.8365 ± 0.1237	0.6570-1.1056	3.87 ± 0.57	42.64
DMDTP	0.1809 ± 0.0575	0.1199-0.2645	1.15 ± 0.37	12.71
DMTP	0.1540 ± 0.0874	0.0657-0.2935	1.09 ± 0.62	11.70
			Total	67.40

It is obvious that on average the highest excreted amount was DCA (837 μg), followed by DMDTP (181 μg), DMTP (154 μg), omethoate (5.2 μg) and Dimethoate (1.9 μg).
The individual results are shown in Appendix 14.1.7.2 (analytical urinary results). Details of the method and the individual data are presented in the specific analytical report by Huntingdon Life Sciences (HLS) (Appendix 14.1.7.3).

9.6 Calculation of excreted amounts relative to the oral dose administered

Calculations were made using the volume (weight) of the respective urine samples multiplied by the achieved concentration of the various compounds to derive the total excreted amount of each compound (mg). Then the total amount of parent and the selected

metabolites excreted was calculated being the sum of all excreted compounds. Thereafter, to calculate the percentages of the total excreted amount of all compounds relative to the oral dosing, all summed figures (mg) were converted to µmol using the respective molecular weight of each compound.

Molecular weights used:

Dimethoate (DM)	: 229.25
Omethoate (OM)	: 213.19
DCA	: 216.21
DMDTP	: 157.17
DMTP	: 141.11

Table 9 summarises the achieved results.

Table 9. Summed excreted total amount of compounds, oral dose and percentages of dose excreted

Subject #	Totally excreted amount (mg)	Totally excreted amount (µmol)	Oral dose Administered (µmol)	% excreted of oral dose
01	1.1134	6.1323	9.15	67.05
02	1.2383	6.4719	9.30	69.56
03	1.2582	6.6204	10.57	62.66
04	1.0685	5.3117	8.04	66.05
05	1.0525	5.4553	8.20	66.53
06	1.3394	6.8683	9.46	72.59
			Mean ± s.d.	67.41 ± 3.37

See also Appendix 14.2.1

The excreted amounts relative to the orally administered dose determined in urine (mean: 64.7 %) are below 100 %, however, the values show to be remarkably consistent among the 6 subjects. Only a 5 % coefficient of variation (3.37/67.41) of the mean value of 67.41 % (s.d. 3.37 %; range 63 – 73 %) is noted among the 6 volunteers investigated

9.7 Calculation of kinetic parameters

All dosing, body weight, urinary data (volume, time of voiding, concentration of urinary compounds etc) and molecular weights, were exported to an Excel spreadsheet developed by TNO and analysed for each subject individually. Using pharmacokinetic methods area under the curves (AUCs), C_{max} and T_{mac} were calculated for each urinary compound and subject (for details see appendix 14.2.1). The main findings are summarised in Table 10.

The pharmacokinetic calculations showed that in all cases excretion of the compounds of the parent (DM) and the primary metabolites (OM and DCA) reached a peak at 2 hours post dosing, which is also true in almost all cases for the compounds DMDTP and DMTP. Furthermore, the excretion of most compounds is completed within 24-28 hours, with the exception for the secondary metabolites DMDTP and DMTP (40-60 h) (See figures in 14.2.1)

Table 10. Pharmacokinetic results of the various excreted urinary metabolites of all subjects

Subj. #	AUC (mg.h/L)					C_{max} (mg/L)					T_{max} (h)		
	DM	OM	DCA	DMDTP	DMTP	DM	OM	DCA	DMDTP	DMTP	DM; OM; and DCA	DMDTP	DMTP
01	0.011	0.1224	5.945	1.716	3.574	0.0035	0.0246	1.50	0.2680	0.4680	2	2	2
02	0.009	0.0232	8.595	2.575	1.230	0.0031	0.0077	2.490	0.4190	0.1920	2	2	2
03	0.011	0.0299	4.460	1.958	2.282	0.0037	0.0073	0.895	0.0972	0.1590	2	2	6
04	0.015	0.0189	7.518	1.307	0.843	0.0048	0.0063	1.820	0.1520	0.1010	2	2	2
05	0.013	0.0151	10.36	4.011	1.222	0.0042	0.0050	2.530	0.5100	0.1520	2	2	2
06	0.012	0.0271	5.159	1.326	1.559	0.0039	0.0065	1.100	0.1660	0.1580	2	6	6

10 Safety results

10.1 Extent of Exposure

In the present study 6 subjects received orally a Dimethoate solution at about 0.03 mg/kg bw. Hereto, a volume of 510-670 µL of a Dimethoate (stock) solution containing on average 3 615 µg/mL was added to 25 mL of demineralised water for each individual.

10.2 Adverse Events

AEs were established by the medical investigator on basis of:
1. Answer to the open question: 'how are you feeling?'
2. Spontaneous reporting
3. Well-being questionnaire (Form P4802 F05; Appendix 14.1.1)

AEs were classified by the medical investigator according to ICD-10, published by the WHO Collaborating Centre for International Drug Monitoring. The medical investigator registered the findings, conclusions and actions according to TNO standard procedures on forms F01 and F02.

In total 2 subjects complained comprising various adverse events.

- Subject 02 suffered from a painful neck, located right which started on 04 August and lasted till 06 August. This complaint was reported as mild and unlikely related to the treatment by the medical investigator. Further, he suffered from a pain left submandibulen lymph node which started 04 August (5 minutes after oral administration), lasted for 5 minutes and ended 04 August. The medical investigator reported this complaint as mild and unlikely related to the treatment. Thirdly, he suffered from a change in bowel habit which started 06 August and lasted till 07 August. This complaint was reported by the medical investigator as mild and unlikely related to the treatment. He lastly complaint about fatigue which started 06 August and continued unknown. This complaint was reported as mild and unlikely related to the treatment
- Subject 05 complaint about fatigue which started on 05 August and ended 05 August. The medical investigator reported this complaint as mild and unlikely related to the treatment.

All above mentioned effects as well as the duration of complaints cannot be explained by the known mechanism of action of Dimethoate.

10.3 Deaths, other serious adverse events and other significant adverse events

None observed

10.4 Clinical laboratory evaluation

As judged by the medical investigator no significant clinically relevant laboratory pre-study, in-study and post-study check up results were reported (see appendix 14.2.5)

10.5 Vital signs, physical findings and other observations related to safety

No abnormal or significant clinically relevant results were recorded in heart rate, blood pressure, and physical examinations in the in-, pre-, and post-study check up Also no clinically relevant ECG or trends in ECG recordings pre-study, and in-study were reported by the cardiologist

10.6 Safety summary and conclusions

Administration of an oral solution of Dimethoate (dose: ~0.03 mg/kg bw) was well tolerated by all subjects in this study. AEs reported, investigations of vital signs and ECG, as well as clinical laboratory parameters were, apart from a few cases, all in the the 'normal range' as expected for healthy subjects. The slightly out of the normal range parameters were all judged as not clinically relevant by the medical investigator. Whether the extreme high environmental temperatures (hot summer) for a very long period during the study play a role in the complaints of fatigue by two subjects is not unlikely.

11 Discussion and conclusions

This report describes the conduct and the results of a study on the single oral administration of Dimethoate and the subsequent urinary excreation of Dimethoate and selected metabolites. The study was designed as a single dose, open study wherein six (6) healthy male volunteers participated with informed consent. In this study approximately 0 03 mg/kg bw was orally dosed per subject (range: 0 0274 – 0.0277 mg/kg bw). Systemic absorption of Dimethoate was evidenced by the establishment of urinary concentrations of Dimethoate, omethoate, DCA, DMDTP and DMTP. All subjects reported their well-being at the end of the study and did not show any signs of adverse health effects.

No post-dose plasma- and RBC-cholinesterase activity levels of any subject did indicate an inhibitory effect, as compared with the average personal 95 %-confidence limit baseline levels. After Dimethoate administration all Che-activities were well within the observed range of biological variation (95 % confidence interval of Che-activity before treatment). Additionally, the pre-dose (t=0 h) and the 4 h post-dose samples revealed no relevant differences and also the overall results of the 0 h, 4 h and 24 h and 72 h samples did not show relevant variations. Due to low variation in the individual baseline values of the subjects, effects, due to Dimethoate treatment, if any, should have been noted under the given test conditions.

Also safety ECG recordings taken various times during the study did not show clinically relevant changes or trends related to the treatment. The same holds for vital sign (blood pressure and heart rate measurements) performed in the study.

In various urine samples of all subjects collected during the study up to 72 hours post dosing Dimethoate, omethoate, DCA, DMDTP as well as DMTP was detected and quantified. Based on calculation of total excreted amounts of all compounds, on average, in ascending order: 1 9 µg Dimethoate, 5.2 µg omethoate, 154 µg DMTP, 181 µg DMDTP and 837 µg DCA has been excreted. When these total amounts of the quantified urinary compounds were converted to µmol and taken relative to the oral administered dose (µmol) of Dimethoate, then an average of 67 4 % ± 3 4; (range 63-73 %) of the administered dose can be recovered by those compounds in the excreted urine under the given test conditions. It is important to note, that potentially tertiary metabolites like dimethylphosphate, monomethylthiophosphate, monomethylphosphate, thiophosphate or phosphate may also be formed from Dimethoate Those compounds cannot be determined sensitively enough in urine samples as they reveal high background levels/variations since they are also formed from intake of natural food/food ingredients. Those metabolites may account for the not quatified amounts in urine Furthermore, a minor extent might also be excreted via faeces. However, based on rodent studies excretion via faeces was almost negligible.

Using pharmacokinetic methods the following kinetic parameters per compound and per subject could be calculated: AUC, C_{max}, and T_{max} From these results it was observed that after an oral dose of Dimethoate, urinary excretion of metabolites reaches a peak (T_{max}) at about 2 hours post dosing for almost all compounds, with the exception of DMDTP and DMTP in subject 06 (6 h) and DMTP in subject 03 (6 h). Further, it has been observed that urinary excretion of almost all compounds is completed within 24-28 h with the exception of DMDTP and DMTP (40-60 h) in some subjects. As the latter two compounds represent secondary or even tertiary metabolites of Dimethoate, the slightly delayed excretion can be explained on the basis of metabolism. The observed time course clearly demonstrates that for the investigated metabolites urine sampling up to a maximum of 72 hours is ample

sufficient to quantify an orally administered dose of Dimethoate, and is in line with the fast excretion noted in rodent studies.

The following conclusions can be drawn from this study:

- An oral administered dose of Dimethoate (~ 0.03 mg/kg bw) is well tolerated by male volunteers;
- No inhibition of RBC and plasma cholinesterase activities was detected after Dimethoate administration as all Che-activities were clearly above the subjects' 95 %-confidence lower limit personal baseline values;
- No clinically relevant changes in ECG or trends in ECG recording could be observed in the study;
- No clinically relevant heart rate, and blood pressure changes could be established;
- Collected urine samples of all subjects comprised all 5 compounds (Dimethoate and 4 metabolites);
- The metabolic profile was very similar among the 6 subjects. Highest amounts of metabolites excreted in descending order are DCA>, DMDTP, DMTP >>, Dimethoate and omethoate;
- Also the total amount excreted was very similar for all 6 subjects. On average 67.4 % ± 3.4 of the orally administered dose was recovered in urine based on the selected compounds quantified in urine;
- The pharmacokinetic calculations showed that in most cases 2 hours post dosing excretion of compounds reached a peak and that excretion of most compounds is completed within 24-28 hours, with the exception of DMDTP and DMTP (40-60 h). Thus, the used urine sampling scheme up to 72 hours is ample sufficient to allow a quantification of an orally administered dose by means of the selected metabolites.
- Based on the obtained results, the determination of the selected compounds in urine provide a suitable tool to measure the systemic absorbed dose e.g., in a field exposure study.

12 Tables and figures referred to but not included in the text

12.1 Summary tables

None

12.2 Figures

None

13 References

1. INO Protocol P4802 "Urinary excretion profile of Dimethoate and its metabolites after single oral administration of Dimethoate in healthy male volunteers" Revised Final 1, dated 12 May 2003.

2. Meuling, W.J.A., M.J.M. Jongen and J.J. van hemmen, An automated method for the determination of acetyl and pseudo cholinesterase in hemolysed whole blood, 1992, Am. J. Ind. Med, 22, 231-241.

14 Appendices

14.1 Study information

14.1.1 Protocol P4802

14.1.2 MSDS and TSDS of Dimethoate

14.1.3 List of IEC/IRB members and letter(s) of approval

14.1.4 Sample informed consent (blank)

14.1.5 CV of principal investigator and medical investigator

14.1.6 Audit certificate

14.1.7 Bio-analytical reports (3)

14.1.7.1 Cholinesterase measurements

14.1.7.2 Urinary and dosing results (Excerpt 14.1.7.3 Huntingdon Ltd. report)

14.1.7.3 Urinary Dimethoate and metabolites analysis report (Huntingdon Ltd.)

14.2 Individual subject data listings

14.2.1 Individual urinary results and calculation (spreadsheets)

14.2.2 Individual cholinesterase assay results (spreadsheets)

14.2.3 Individual ECG results

14.2.4 Individual administration of Dimethoate (spreadsheet)

14.2.5 Individual clinical laboratory data (screening, in-study and post-study)

14.3 Case Report Forms

Not applicable

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TNO Report

V4802 CONFIDENTIAL| Final |

APPENDICES: PART A
Urinary excretion profile of Dimethoate and its metabolites after single oral administration of Dimethoate in male volunteers

Date	28th December, 2004
Authors	W J A Meuling, BSc and L. Roza, PhD
Sponsor	Dimethoate Task Force (DTF), Wotanstrasse 39, D-68305 Mannheim, Germany
TNO project number	010.44162
TNO study code	4802
Sponsor's study code	104-084
Status	Final, 28 December 2004
Number of pages Part A	227
Number of pages (total)	439

Appendix 14.1.1. Protocol P4802: Urinary excretion profile of dimethoate and its metabolites after single oral administration of dimethoate in healthy male volunteers
(117 pages this page excluded)

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TNO Protocol

P 4802

Urinary excretion profile of dimethoate and its metabolites after single oral administration of dimethoate in male volunteers

Date	12 May, 2003
At the request of	Dimethoate Task Force, Wotanstrasse 39, D-68305 Mannheim, Germany
Drafted by	W.J.A. Meuling, BSc L. Roza, PhD
TNO project number	010.44162/01.01
TNO study code	4802
Status	Revised Final 1, 12 May 2003
Previous versions	Final, 08 January 2003 Draft 4, 16 December 2002 Draft 3, 05 December 2002 Draft 2, 25 November 2002 Draft 1, 12 November 2002
Number of pages	117 (including appendices)
Number of appendices	08 Appendices 10 Forms

Contents

1 List of Abbreviations and definitions

ADI	:	Acceptable Daily Intake
AE	:	Adverse Event
ANOVA	:	Analysis of Variance
ARfD	:	Acute reference dose
BMI	:	Body Mass Index
BP	:	Blood Pressure
BPM	:	Beats Per Minute
BW	:	Body weight
CAS	:	Chemical Abstract Services
CofA	:	Certificate of Analysis
CRO	:	Contract Research Organisation
DCA	:	Dimethoate carboxylic acid (Dimethoate metabolite)
DTF	:	Dimethoate Task Force
DTP	:	Dithiophosphate (Dimethoate metabolite)
ECG	:	ElectroCardioGram
GCP	:	Good Clinical Practice
GLP	:	Good Laboratory Practice
HR	:	Heart rate
ICD-10	:	International Statistical Classification of Diseases and Related Health Problems
ICH	:	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
LC-MS	:	Liquid Chromatography-Mass Spectrometry
METC	:	Medisch Ethische ToetsingsCommissie (Medical Ethics Committee)
NOAEL	:	No Observed Adverse Effect Level
OECD	:	Organisation for Economic Cooperation and Development
QAU	:	Quality Assurance Unit
RBC	:	Red Blood Cell
Retics	:	Reticulocytes
SAE	:	Serious Adverse Event
SCC	:	Scientific Consulting Company
SOP	:	Standard Operating Procedure
SSDZ	:	Diagnostisch Centrum SSDZ; (Stichting Samenwerkende Delftse Ziekenhuizen)
TNO	:	Nederlandse organisatie voor Toegepast Natuurweten-schappelijk Onderzoek (Netherlands Organization for Applied Scientific Research)
TP	:	Thiophosphate (Dimethoate metabolite)
TSDS	:	Technical Safety and Data Sheet
WBC	:	White Blood Cell
WHO	:	World Health Organisation

2 Synopsis

Title	:	Urinary excretion profile of dimethoate and its metabolites after single oral administration of dimethoate in healthy male volunteers
Sponsor		
- Name and address	:	Dimethoate Task Force (DTF), Wotanstr 39, D-68305 Mannheim, Germany
- Responsible person	:	Mr Peter Hofmann
Study monitor		
-Name and address	:	SCC Scientific Consulting Company Mikroforum Ring 1, D-55234 Wendelsheim, Germany
-Contact person	:	Werner Köhl, PhD
CRO		
- Name and address	:	TNO Nutrition and Food Research, Department of Target Organ Toxicology, P.O.B. 360, 3700 AJ Zeist, The Netherlands
- Study code	:	4802
- Principal Investigator	:	W.J.A. Meuling, BSc
Design	:	A controlled, single dose, open study
Objectives	:	To establish in healthy male volunteers the urinary elimination profile of dimethoate and/or its metabolites after a single oral administration of dimethoate
Test substance		
- Name	:	Dimethoate
- Aim	:	Organophosphorous pesticide
- Galenic form	:	White powder
Study treatments		
-Oral administration	:	Dimethoate in distilled water
-Dosage	:	0.03 mg/kg b.w.
Participants		
- Description	:	Male volunteers
- Number	:	6
- Inclusion criteria	:	Age ≥18 years ≤ 45 years on Day 01
	:	BMI >22 - <30 kg/m^2
	:	Healthy as assessed by health questionnaire, physical examination and clinical chemistry (fasting state) including: haematology (RBC, WBC, platelets, Ht, Hb, Retics) and WBC

blood differentiation; Clinical chemistry profile (ALT, ALP, AST, γ-GT, albumine, total bilirubin, creatinin, glucose and urea); Drug screen in urine (methadon, benzodiapines, cocaine, amphetamine/methamphetamine, opiaten, tetrahydrocannabiol, barbituraten, tricyclische antidepressiva)
Dipstick urinalysis (protein, glucose, leucocytes, erythrocytes, nitrite, pH, ketones, urobilinogen, bilirubin); if the dipstick test gives values above the normal range for leucocytes, blood or protein, a microscopic inspection of urine sediment will be done

: Having given their written informed consent
: Voluntary participation
: Willing to comply with the study procedures
: Willing to refrain from blood donation 30 days in advance of Day 01 and during the whole study
: Willing to accept use of all anonymized data, including publication, and the confidential use and storage of all data
: Willing to accept the disclosure of the financial benefit of participation in the study to the authorities concerned
: Willing to refrain from intake of nutritional supplements during a period of 2 weeks prior to the study and ending at 72 h after the oral administration in the study

- Exclusion criteria:

: Participation in any clinical trial including blood sampling and/or administration of substances up to 90 days before Day 01 of this study
: Under medical treatment of a specialist in the past and present for a reason that may affect the study outcome
: Use of prescribed medication (paracetamol excluded)
: Alcohol consumption more than 28 units/week(1 unit of alcohol equals 10 grams of ethanol)
: Occupational exposure to, contact with, or use of agrochemicals
: Physical training exceeding 10 hours of heavy weekly training or planning to train for an event requiring extreme physical training like triathlon or marathon
: High background levels in two blank urines of the urinary metabolites to be established, collected within three weeks prior to Day 01

	: Large variation (C.V. >15%) of plasma and RBC cholinesterase activities of three blank samples collected within three weeks prior to Day 01
	: TNO personnel and their relatives in the first and second remove
	: Having unsuitable veins for blood sampling
	: Not having a general practitioner
	: Not willing to accept information-transfer concerning participation in the study, or information regarding his health, like laboratory results, findings at anamnesis or physical examination and eventual adverse events to and from his general practitioner or his objection concerning participation
	: Not willing to accept transfer of medical information to and from the subjects' general practitioner when results of the study urgently demand for it with respect to the health of the subject
Study treatment	
- Oral administration	: The test substance will be dissolved in distilled water just prior to the administration
- Dosage	: 0.03 mg/kg b.w.
- Conditions exposure	
- temperature	: Ambient
- relative humidity	: Ambient
Safety parameters	
- Anamnesis	: Health and life style questionnaire (pre-screen)
- Vital signs	: BP and HR (pre-screen, pre-dose, 3h, 5h, and 24h post dosing)
- Adverse events	: On all study days (immediately record time effects first occur)
- Physical examination	: Pre- and post study screening
- Clinical chemistry	: Pre-screen, pre-dose, post dose (24h) and post study screen (72h)
- Haematology	: Pre-screen, pre-dose, and post study screen (72h)
- Urinalysis	: Pre-screen, pre-dose, and post study screen (72h)
- ECG	: Pre-screen, pre-dose, post dose (3h and 24 h)
- Drug screen in urine	: Pre-screen
- Virology	: Not applicable
Study parameters	
- Kinetics	: Concentration time course of dimethoate,

	omethoate, DCA, DTP and TP in urine after oral administration. Also expressed corrected for creatinin content.
- Dynamics	: RBC and plasma cholinesterase activity measurement
- Additional tests	: Body weight (pre dose)
Sample collection	
- Blood (pre-study)	: pre-study (Day -18, -14, -11) (3 samples per subject)
- Blood (in study)	: pre-dose, 4h, 24h and 72h post dosing (4 samples per subject; totally 24 samples)
- Urine (pre-study)	: pre-study (Day -15-14; 24h, and Day -12-11; 24h) (2 samples per subject)
- Urine (in study)	: blank, 0-4h, 4-8h, 8-12h, 12-24h, 24-36h, 36-48h and 48-72h post dosing (8 samples per subject; totally 48 samples)
- Dosing solution	: Directly after administration
Sample analysis	
- Urine	: Dimethoate, omethoate, DCA, DTP, TP and creatinin content
- Blood (hemolyzed)	: Plasma and RBC cholinesterase activity
- Dosing solution	: Dimethoate
Study period	
- Duration	: 4 study days (pre-exposure period excluded)
- Lodging	: Not applicable
- Visits to TNO	: Pre-study screening, and seven study days
Restrictions (in study)	
- Life-style	: Fasting from 22:00 hours the night before until sampling the next morning (Day 01, Day 02, Day 04) Abstain from smoking 2 hours prior to dosing until 6 hours post dosing on Day 01
- Diet	: Abstain intake of nutritional supplements during a period of 2 weeks prior to the study and ending at 72 h after the oral administration in the study
Statistical analysis	
- Anthropometry	: Descriptive
- Kinetics	: Mean and standard deviations will be calculated
Responsibilities	
- Drafting protocol	: TNO Department of Nutritional Physiology
- Drafting study forms	: TNO Department of Nutritional Physiology

- Test substance
  - Supply : Sponsor
  - Coding : Sponsor
  - Characterisation : Sponsor, Certificate of Analysis (CofA) & TSDS included
  - Liability : Sponsor
- Insurance (subjects) : Sponsor delegated insurance to TNO
- Clinical chemistry/Haematology: TNO Department of General Toxicology
- Cholinesterase activities : Diagnostisch Centrum SSDZ
- Chemical analysis
  - urinary metabolites : Huntingdon Life Sciences Ltd, Suffolk, U K.
  - dosing solution : Huntingdon Life Sciences Ltd, Suffolk, U K
  - urinary creatinin : TNO Department of General Toxicology
- Kinetics : Department of Nutritional Physiology
- Statistical analysis : Department of Nutritional Physiology
- Drafting report : Department of Nutritional Physiology
- Study auditing : TNO Quality Assurance Unit, TNO Nutrition and Food Research
- Study monitoring : SCC Scientific Consulting Company

Tentative time schedule

- Approval Medical Ethics Committee (TNO-METC) : 14 March 2003
- Start recruitment subjects : After written approval of TNO-METC and availability of stability data of the metabolites in urine; week 23
- Inclusion of subjects : Within one week after receipt of preselection results of plasma and RBC cholinesterase activities and urinary metabolite levels; week 31
- Substance supply to TNO : At least one week prior to the start of clinical part
- Study initiation clinical part : August 2003; week 32
- Completion clinical part : Within one week after initiation of the clinical part; week 32
- Receipt audited analysis data : Within 10 weeks after completion of clinical part; week 42
- Draft final report (unaudited) : Within 2 weeks after receipt audited analysis data; week 44
- Final report (audited) : Within 2 months after approval of draft final report by the sponsor

3 Schedule of assessments

The proposed schedule of assessments is given below.

Activity	Study Day*								
	≥-21	-18	-14	-11	-04	01	02	03	04
Candidates information (oral)	+								
Pre-study screen (fasting)									
- Health questionnaire	+								
- Anamnesis	+								
- Physical examination	+								
- Vital signs	+								
- ECG	+								
- Blood sampling	+								
- Urine collection	+								
Pre-selection									
- Blood sampling		+	+	+					
- Urine collection			+	+					
Selection subjects									
Eligibility and inclusion					+				
Allocation						+			
Well-being questionnaire						+	+		+
(Serious) Adverse Events						+	+	+	+
Treatment									
- Body weight						+			
- Oral dosing (dimethoate)						+			
- Blood sampling						+	+		+
- Urine collection						+	+	+	+
- Vital signs (BP)						+	+		
- ECG						+	+		+
Post study screen (fasting)									
- Blood sampling									+
- Urine collection									+
- Physical examination									+
- ECG									+
- Exit interview									+

* Day 01 is defined as the first day of the study the treatment (oral administration) will take place. A planned order of treatment is given in this schedule. Due to logistic reasons minor changes in planning may take place. In the final report the actual treatment order will be reported

4 Responsible personnel and test facilities

4.1 Sponsor

Dimethoate Task Force (DTF),
Wotanstrasse 39,
D-68305 Mannheim,
Germany.

Contact person at DTF

Mr Peter Hofmann

Study monitor for DTF

Scientific Consulting Company (SCC),
Mikroforum Ring 1,
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Germany

Contact person at SCC

Werner Köhl, PhD
Phone : +49 6734 919 0
Fax : +49 6734 919 191
Email : Werner.koehl@ SCC-gmbh.de

4.2 Testing facilities

TNO Nutrition and Food Research
Utrechtseweg 48
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Fax : +31 30 69 57 22 4

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4.3 Facility for the cholinesterase analysis

Diagnostisch Centrum SSDZ (Stichting Samenwerkende Delftse Ziekenhuizen)
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Fax : +31 15 256 81 03

Responsible person at SSDZ
Teamleader Chemistry Unit
H. Bekenes
Phone : +31 15 260 45 23
Fax: : +31 15 256 81 03
Email : Bekenes@RDGG.nl

4.4 Facility for chemical analysis (urine and dosing solution)

Analysis of urine metabolites and dosing solutions will be carried out under the responsibility of the sponsor by the following selected GLP certified laboratory.

Huntingdon Life Sciences Ltd,
Eye
Suffolk, IP23 7PX
United Kingdom

Responsible person at Huntingdon Life Sciences Ltd
Study manager (Residue Analysis, ERC)
H Harper
Phone : +44 1379 67 21 72
Fax: : +44 1379 67 21 00
Email : harper@ukorg.huntingdon com

4.5 Reponsibilities

The sponsor will be responsible for the financial compensation for the conduct of the study. The sponsor will be liable for the test substance, for the prompt delivery to TNO and will be responsible for arranging and delivery of detailed information regarding the description of the test substance including a certificate of analysis. Further the sponsor is responsible for the analytical determination of dimethoate, omethoate, dimethoate carboxylic acid (DCA), dithiophosphate (DTP), and thiophosphate (TP) in urine and dimethoate in the dosing solution according to validated analytical procedures by a GLP certified laboratory (Huntingdon Life Sciences Ltd) and for the prompt forwarding of audited analysis results to TNO for inclusion in the (draft) final report.
At the request of the sponsor the responsibility for an insurance for the subjects for damage or death caused by their participation in this study (the pre-study selection included) according to Dutch law "WMO", has been delegated to TNO. Insurances for material damage and accidents during the travel to and from TNO and during the stay at TNO are the responsibility of TNO

W.J.A. Meuling will be responsible for the actual oral administration and the overall conduct of the study. He will be responsible for the dispatch to the chemical laboratory (Huntingdon Life Sciences Ltd) of the dosing solution and frozen urine samples and for the dispatch of hemolyzed blood samples to the clinical chemistry laboratory (SSDZ)

I. Roza will be responsible for the overall conduct of the study in case Mr. W.J.A. Meuling is not available.

W.A.A. Klöpping-Ketelaars will be responsible for the selection of the subjects, medical aspects of the study, the documentation, the interpretation and the reporting of the AEs and SAEs. Parts of the screening may be delegated to an assistant medical investigator appointed by the management

F.W. Sieling will be responsible for the daily conduct of the clinical part of the study, which includes the pre- and post screening, ECG measurements, the allocation of the subjects to entry-numbers, the collection of blood samples, the receipt of collected

urine samples, the measurement of weight, the storage of blood/plasma and urine samples, and the daily contacts with the subjects The direct involvement in the daily conduct of the clinical part will be delegated to qualified personnel appointed by the management

H. Bekenes, will be responsible for the enzymatic determination of plasma and RBC cholinesterase in hemolysed blood samples. The direct responsibility will be delegated to qualified personnel appointed by the management.

R.A. Woutersen, will as head of the Department of General Toxicology be the overall responsible person for the clinical chemistry, the drug screen and haematology analysis in blood and urine and for the analytical determination of urinary creatinin content The direct responsibility will be delegated to Mr. J.F. Catsburg

5 Introduction

5.1 General

In agriculture workers may be exposed either dermally, inhalatory and/or orally to dimethoate by performing different tasks. However, knowledge about the systemic absorption and urinary excretion profiles of subjects is very limited. This study is undertaken to investigate the urinary excretion profile of dimethoate and/or its metabolites after a single oral administration of dimethoate. The results of this study will be used to investigate the exposure of agricultural workers (operators) to dimethoate in typical field exposure scenarios.
The present study protocol has been drafted in accordance with, and the study will be conducted according to the ICH Guidelines for Good Clinical Practice (ICH Topic E6 (Guideline for Good Clinical Practice) adopted 01-05-1996 and implemented 17-01-1997.

5.2 Toxicological profile of dimethoate

General
Dimethoate (CAS no. 60-51-5) is an organophosphate insecticide. It is used against a wide range of insects, on ornamental plants, fruits, and vegetables. It is also used as a residual wall spray in farm buildings for house flies. Dimethoate has been administered to livestock for control of botflies.

Fate of dimethoate in humans and animals [1]
The metabolism profile is not affected by route of administration, sex, or dose level. Dimethoate is shown to be rapidly and quantitatively absorbed after oral administration. The substance is extensively metabolised resulting in the main metabolites dimethoate carboxylic acid (DCA), dithiophosphate (DTP), thiophosphate (TP) and phosphate methyl esters in the rat. Formation of the oxygen analogue of dimethoate, omethoate, is a minor pathway of the metabolism. Omethoate is further

metabolised in the same way as dimethoate The excretion of dimethoate and corresponding metabolites is very efficient and mainly within urine (80 - 90 % of the applied dose excreted within 24 hours after oral or i.v. administration). There was no tendency for accumulation in any tissue.
The dermal absorption was intensively studied in vivo and in vitro to allow a refinement of human (operator) risk assessment after dermal exposure. From the in vivo study in rats absorption rates for typical use conditions, handling of undiluted material (400 g/L EC) and exposure to the 1/200 representative spray-strength dilution of 6 % and 24 %, respectively, were deduced. In addition, an in vitro penetration study revealed significant differences between rat and human skin penetration of dimethoate. Based on mean percentage absorption after 8 h a 22 fold and 5 fold higher absorption rate for rat skin in comparison to human skin were deduced for the concentrate (400 g/L EC) and the 1/200 spray-strength dilution, respectively Consequently, the extrapolation from findings in rat studies after oral administration to assess potential effects after dermal exposure in humans can be refined. Based on the dermal absorption data in vivo in the rat and taking into account the ratio observed between human and rat in the comparative in vitro dermal penetration study the following dermal absorption values for concentrate and spray dilution can be deduced. For the concentrate 0.5 % (0.27 % based on the experimental findings: 6 % / 22 = 0.27 %) and for the spray-strength dilution 5 % (4.8 % based on the experimental findings: 24 % / 5 = 4 8 %) of an external dose respectively, might become bioavailable. These dermal absorption values are used in the operator risk assessment

Acute effects
Dimethoate is of moderate toxicity after acute administration to rats and mice and has to be labelled with R 22 "harmful if swallowed". The LD50 being 358 in male and 414 mg/kg bw in female rats, and 160 mg/kg bw in male and female mice. The signs of toxicity were those typical of cholinesterase inhibition. Dimethoate is of low toxicity after dermal administration, the LD50 being > 2.000 mg/kg bw. Body tremors and abnormal gait as well as reduced bodyweight gain in week 2 were the only signs observed

Dimethoate is non-irritant to the skin and eyes of rabbits. In a Buehler-type sensitisation test, dimethoate was found to have no sensitising potential.

Short-term toxicity
Minor reductions in bodyweight gain and food consumption were observed in short-term studies at dietary concentrations 100 ppm. Apart from inhibition of cholinesterase activity, dimethoate had no effect on the blood or urine composition. The liver and kidney weights of animals tested at the higher doses (100 ppm) tended to be lower than those of the control groups; there were however, no microscopic changes, and the effect is unlikely to be of toxicological significance.

Cholinesterase inhibition was the only toxicologically relevant effect noted at lower doses (< 100 ppm). The NOAELs were thus generally based on reductions of cholinesterase activity in the relevant compartments erythrocytes or brain

With respect to the NOAELs obtained in the short-term dietary toxicity studies it must be recognised that in rats the available studies were range-finders or relatively old studies with a partially limited scope of investigations Therefore, the relevant NOEL for short-term toxicity in rats has been derived from the 28-day study which was similar to OECD 407 instead using the data from the available 90-day study, although the NOELs are quite similar for both studies

Following repeated dermal application of up to 1000 mg/kg bw/day on intact or abraded skin of rabbits for 21 days, no treatment related adverse systemic effects or effects at the site of application were noted

Mutagenicity/Carcinogenicity

The submitted full reports of mutagenicity studies (with the exception of an supplementary Ames test) were conducted with a batch of dimethoate (611A) which corresponds closely with the technical specification of the dimethoate currently produced by the Joint Submission Group and are considered relevant for the evaluation This is essential in the context of the evaluation of published results in the literature, where often unstated qualities or "crude" material is used

The submitted unpublished studies provide some evidence for dimethoate being weakly genotoxic in vitro (positive in Ames test and in liver UDS assay) but not in vivo A review of the literature relating to the mutagenic potential of dimethoate revealed a number of positive results with dimethoate of unspecified purity, notably in additional bacterial assay using different S typhimurium strains and in some indicator tests in which the genotoxic potential was investigated by means of sister-chromatid exchanges in fish and mammalian cells in vitro

In contrast only negative results were obtained in all submitted unpublished studies in somatic or germ cells in vivo, i e a rat bone marrow cytogenetics assay, a mouse bone marrow micronucleus assay, a rat liver UDS assay and a mouse dominant lethal assay

In published in vivo studies with somatic cells study authors concluded to show positive effects for dimethoate especially with regard to chromosomal damage However, the findings of these studies are regarded as inconclusive with respect to predicting the genotoxic potential of dimethoate produced by the Joint Submission Group because of the lack of information on the purity of the dimethoate tested, the limitations in study design and/or the parameters investigated (e g reduction in chromosome number in mice studies)

The reported Comet assay in vivo revealing DNA-damage in all investigated tissues to a quite similar extent was considered not relevant, due to limitations in the used methodology Furthermore, the observed tissue distribution can hardly be explained by the known toxicokinetic of dimethoate In face of all these facts and as in vivo mutagenicity assays investigating biologically more relevant endpoints (UDS and micro nucleus test), which were conducted with the dimethoate batch 611A and

which showed no positive findings, the Comet assay data is considered to be not relevant.

None of the reported positive findings in publications were noted in any of the submitted guideline compliant studies with the dimethoate produced by the Joint Submission Group and therefore, they are not considered to be relevant for the hazard characterisation with regard to mutagenicity in somatic cells. In addition, no indication for a carcinogenic potential of dimethoate was observed in any of the available carcinogenicity studies.
Among the eight in vivo studies investigating genotoxic effects in rodent germ cells, a positive response has been reported in two older, poorly documented studies. In both studies material of unknown purity (e.g. „industrial crude product") was used. Based on the negative findings in the submitted study with defined material and the published studies with pure dimethoate revealing no genotoxic effects in germ cells, the positive findings are not considered to be relevant for assessing the genotoxic potential of dimethoate produced by the Joint Submission Group.

Thus, based on the negative findings in the relevant in vivo studies using dimethoate with technical specifications as currently manufactured by Joint Submission Group members, no indications exist that this material might have genotoxic potential in somatic or germ cells.

No carcinogenic potential is associated with dimethoate based on the valid lifetime studies in rats and mice. Again, cholinesterase inhibition was demonstrated to be the primary and most sensitive parameter affected after exposure whereas other effects (retarded growth, anaemia, leukocyte counts, spleen, liver, ovary weights with no histological correlate) were observed at the highest dose level only. These findings did not indicate a specific mode of action or reveal a specific target organ for toxicity apart from cholinesterase inhibition. These other findings can be classified either as unspecific and/or being secondary effects following a biologically relevant inhibition of cholinesterase activity

In a two year feeding study in rats, other effects like reduced bodyweight gain, increase in leukocyte counts, anaemia, increase in spleen weight and reduction in ovary weights were observed only at the highest dose level of 100 ppm. A marked inhibition of cholinesterase in plasma, erythrocyte and brain was also observed. At the intermediate dose of 25 ppm only inhibition of cholinesterase activity in erythrocyte and brain was found whereas no changes of toxicological significance were seen at 5 ppm or 1 ppm.

25 ppm corresponding to 1.16 and 1.48 mg/kg bw/day in males and females, respectively, was considered to be a LOAEL based on cholinesterase inhibition.
A NOAEL of 5 ppm dimethoate in the diet, which is equivalent to 0.25 to 0.3 mg/kg bw/day was deduced from this rat study.

In mice fed a diet containing 0, 25, 100 or 200 ppm Dimethoate for 18 month, retarded bodyweight gain, increased liver weight as well as a hepatocyte vacuolisation and extramedullary haematopoiesis in males only were observed at the

highest dose level. Plasma and erythrocyte cholinesterase inhibition was observed at 200 and 100 ppm. In addition, increased liver weight and hepatocyte vacuolisation was noted in the intermediate dose. Slight decrease in erythrocyte cholinesterase activity at 25 ppm was considered to be an early sign of a potentially adverse effect. Thus, a clear NOAEL cannot be deduced from this study (results for ChE-inhibition indicate a NOAEL of < 25 ppm dimethoate in the diet equivalent to 3.6 and 5.2 mg/kg bw/day for males and females, respectively). However, the observed dose-response relationship, and the extent of inhibition seen at 25 ppm indicate, that the real NOAEL will be close to the lowest dose tested in this study and thus well above the findings in the corresponding rat study.

Reproductive Toxicity

The potential effects of dimethoate on reproduction and development were studied in multi-generation studies in rats and mice and in teratogenicity studies in rabbits and rats.

Two multi-generation studies in mice are available for dimethoate. In the one performed with the material produced by the Joint Submission Group no evidence of reproductive toxicity was seen up to dietary dose levels of 50 ppm (about 15 mg/kg bw /day). Thus, a NOEL for reproductive performance of 50 ppm equivalent to 13.6-15.3 mg/kg bw/day was deduced. In a separate poorly documented drinking water study reported in the literature a single dose of 9.5 -10.5 mg/kg bw/day induced adverse reproductive effects, including reduced mating success, reduced number of developing follicles in ovaries (but no effects on the testes), and increased pup mortality. In both studies cholinesterase activity, as the most sensitive parameter for adverse effects induced by dimethoate, was not determined. Thus, a reliable NOEL for maternal toxicity cannot be deduced for both studies. A marked inhibition of cholinesterase activity can be expected in the dams at the high dose levels applied in both studies, based on the findings in other studies.

A detailed and guideline compliant two generation dietary reproduction study was also performed for dimethoate in rats.

Dimethoate was administered at dietary levels of 1, 15 and 65 ppm. A marked inhibition of cholinesterase activity in plasma, erythrocytes and brain in both generations and slightly reduced bodyweight gain were observed in parental animals at 65 ppm whereas only brain and erythrocyte cholinesterase was inhibited with 15 ppm dimethoate. Although the original report of the study concluded that reproductive effects were found at 65 and 15 ppm, the independent review of the data by US-EPA (1993) and JMPR/WHO (1996) considered that there were no treatment or compound related effects on reproduction at the mid dose of 15 ppm. Recently, the MAFF (Ministry of Agriculture, Food and Fishery) also concluded in 2001 that the study is not showing evidence of reproductive effects at 15 ppm taking historical control data into account.

The NOEL for parental toxicity found in this study is 1 ppm corresponding to

approximately 0.08 mg/kg bw/day based on cholinesterase inhibition at the mid dose level. No evidence for a substance-related adverse effect on pregnancy rate is observed at 15 ppm. Therefore, the reproductive NOAEL is considered to be 15 ppm, equivalent to 1.2 mg/kg bw/day based on reductions in pregnancy rate, litter size at birth and mean pup weight at day 21 at the high dose level of 65 ppm. At this dose substantial ChE-inhibition was noted. Thus, the observed effects on reproduction might be secondary to general toxic effects.

The NOEL of 0.08 mg/kg bw/day for parental toxicity is rather low when compared to the results from long-term studies, where the NOEL is shown to be about 0.23 mg/kg bw/day also due the ChE-inhibition at the higher dose level. The lower NOEL of 0.08 mg/kg bw/day determined in the 2-generation study compared to other studies is most likely due to the dose selection in this study. Therefore, the NOEL for long-term exposure of 0.23 mg/kg bw/day is considered as the relevant value to be used for the risk assessment of potential chronic effects of Dimethoate in humans.

Two valid developmental toxicity studies in two species (rabbit and rat) showed no selective developmental toxicity and no embryotoxic or teratogenic effects. In addition no evidence for teratogenic activity was noted in the published studies in mice and rats
In rabbits a NOAEL for maternal toxicity was 10 mg/kg bw/day and for foetotoxicity 20 mg/kg bw/day, both based upon a slight reduction in bodyweight gain at higher dose levels of 20 and 40 mg/kg bw/day, respectively.

Rats showed maternal toxicity (reduced bodyweight gain and food consumption, body tremor, hypersensitivity and abnormal gait) at the highest dose level of 18 mg/kg bw/day. In this study dimethoate did not cause any embryotoxic, foetotoxic or teratogenic effects resulting in a NOAEL of ³18 mg/kg bw/day. A NOAEL for maternal toxicity was determined at 6 mg/kg bw/day in face of the effects at the highest dose level.

There are no indications, that dimethoate causes selective reproductive toxicity or induces developmental malformations based on the findings in the available studies.

Human data
Occupational exposure to dimethoate, principally through inhalation and dermal exposure, may occur during its manufacture, formulation, and use, and cases of poisoning as a result of accident or neglect of safety precautions have been reported. The oral lethal dose for human beings has been estimated to be in the range of 50 to 500 mg/kg bw.

There are human data available, which are of great importance for the hazard and risk assessment of dimethoate in humans

In a comprehensive study with human volunteers (men and women) the effects of different doses of dimethoate (7, 21, 42, 63, and 84 mg/person/day) administered for 5 days per week for a total of 39 days were investigated. In this study, the most

sensitive and critical effect of dimethoate, the effect on cholinesterase, was investigated.

No gastrointestinal or other clinical effects beside cholinesterase inhibition measured in whole blood and in erythrocytes was noted up to highest dose tested.

A toxicologically significant inhibition in cholinesterase activity in whole blood was noted at oral administration to 0 6 mg/kg bw/day and above, but essentially no effect at doses up to 0.3 mg/kg bw was noted. At the higher dose levels cholinesterase inhibition was observed at an earlier stage and to a somewhat faster rate than in the 0.6 mg/kg bw/day group
This human study demonstrating a clear dose-response relationship for the most sensitive parameter of dimethoate toxicity allows a scientifically sound NOEL determination for humans after oral application of dimethoate and is therefore used as the most relevant basis for ARfD, AOEL and ADI deduction, especially, as none of the available animal studies with dimethoate indicates a specific hazard like developmental, reproductive or mutagenic/ carcinogenic hazard.

A daily dose of 0 3 mg/kg bw/day given to human volunteers of both sexes 5 days a week for 39 days can be regarded as a NOEL. For single and short-term oral administration, therefore, the application of a NOAEL of 0.3 mg/kg bw/day seems to be justified since no effects were noted after repeated application for up to 39 days of this dose.

It seems more appropriate to rely on mean daily doses (average exposure 7 days/week based on 5 days with and 2 days without dosing) for the deduction of a mid- to long-term oral NOEL. Thus, for the longer exposure scenarios a NOEL of 0.2 mg/kg bw/day (0.3 x 5/7 = 0.21) can be considered as a basis for extrapolation for both sexes.

For dimethoate a comprehensive package of acute, short-term and long-term toxicity studies, mutagenicity, carcinogenicity, reproduction/developmental toxicity and neurotoxicity studies is available. For dimethoate neither a mutagenic nor a carcinogenic potential has to be expected. No selective developmental toxicity has been reported. In addition, there is no indication, that dimethoate might cause selectively reproductive toxicity. The inhibition of cholinesterase represents the primary and most sensitive target for dimethoate effects. Other effects than cholinesterase inhibition are of secondary nature and can be considered to be closely related to the primary mode of action, i.e. inhibition of cholinesterase activity above a trigger value were it becomes biologically relevant.

5.3 Acute reference dose [1]

Based on toxicological results a so-called acute reference dose has been calculated. As dimethoate is neither teratogenic, mutagenic, carcinogenic nor a reproduction toxin, the human data (investigating the most sensitive parameter for a toxic response

the cholinesterase inhibition for an exposure of 39 day) is considered as a very conservative basis to deduce the acute reference dose (ARfD). A no effect level of 0.3 mg/kg bw/day was deduced from a human study. Using an assessment factor of 10 for interindividual variations, and the relevant NOEL of 0.3 mg/kg bw/day, the ARfD is calculated to be: ARfD = 0.3 mg/kg bw/day : 10 = 0.03 mg/kg bw/day
The same acute reference dose was proposed by MAFF in 2001.
This assessment is further supported by the findings in animal studies. In an acute neurotoxicity study (special feeding protocol) with additional investigations of cholinesterase inhibition a NOAEL of 2 mg/kg bw was obtained. Applying an assessment factor of 100 to this finding would result in an ARfD of 0.02 mg/kg bw/day which is very similar to the one deduced from the human data.

5.4 Rationale for this study

Once knowledge on the relationship between the level of exposure and the systemic absorption has been gained, the risk for agricultural workers using this substance can be assessed properly.
This study is set up to:

- establish the urinary excretion profiles in man after oral administration of dimethoate;
- establish plasma and RBC cholinesterase activities, which are considered to be biological monitoring parameters

The treatment will be conducted in 6 subjects in such away that to every subject about 0.03 mg/kg b.w. dimethoate will be administered orally. The subjects in this study will be exposed to dimethoate only once.

6 Objectives of the study

The objective of the present study is to establish in healthy male volunteers the urinary elimination profile of dimethoate and/or its metabolites after a single oral administration of dimethoate.

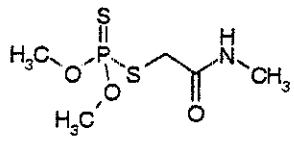
7 Study design

The study is designed as a controlled, single dose, open study

8 Study substance and study treatment

8.1 Description of study substance

- Common name	:	Dimethoate
- Chemical name	:	*O,O*-Dimethyl *S*-methylcarbamoylmethyl-phosphorodithioate
- CAS registry number	:	60-51-5

- Aim	:	insecticide
- Molecular formula	:	$C_5H_{12}NO_3PS_2$
- Molecular weight	:	229.3
- Structural formula		
- Galenic form	:	white powder
- Water solubility	:	25 g/l (21°C)

Relevant toxicological properties

ARfD (Acute reference dose) : 0 03 mg/kg b w /day

The test substance (technical active ingredient) will be provided by the sponsor. A Certificate of Analysis (CofA) and the Test Substance Data Sheet (TSDS) will be included.

8.2 Labelling

At arrival of the test substance at TNO (test substance custody department) the date of arrival, and other specific information will be registered and a study substance code will be generated The test substance will be labelled with a preprinted label, containing the TNO study number (4802) and the study substance code in addition to the original label.

8.3 Storage conditions

The test substance will be kept at storage conditions at +2°C to +10° C in a refrigerator. The test substance will not heated up/warmup above 25°C and will not applied to alkaline and strongly acidic condition and stored in a locked and secured storage facility accessible to authorized personnel only.

8.4 Remainder of study substance

At the termination of the study a representative part of unused study material will be archived for 5 years if the nature of the substance allows it. After expiration of the above indicated storage time period the sponsor will be consulted and will in writing indicate the future of the stored material.

8.5 Lodging

For this study lodging will not be neccesary. Subjects will only be confined to the TNO Clinical Trial Unit during the oral administration on Day 01 and the consecutive blood and urine sampling thereafter up to about 6 hours post dosing.

8.6 Blinding and unblinding

Since this study is an open study, blinding or unblinding is not applicable.

8.7 Study approach

In this study the following general approach will be used:
In each subject the individual urinary excretion profiles of dimethoate and /or its metabolites will be established after a single oral administration of dimethoate followed by consecutive collection of all produced urine, fractionated, up to 72 hours post dosing.
At predetermined time intervals the activities of plasma and RBC cholinesterase will be established in hemolysed blood up to 72 hours post dosing

8.8 Study treatment

8.8.1 Oral administration

To each subject dimethoate in water will be administered at a dose level of about 0.03 mg/kg b.w.
Preparation of the study treatment solution:
About 48 mg dimethoate will be weighed and dissolved in 12 ml distilled water. This stock solution will be used for the individual administrations and contains about 4 mg dimethoate per ml solution. Based on the individual body weights of the subjects, from this stock solution per subject about 500-700 µl will be added to a glass cup containing about 25 ml distilled water and gently homogenised. This amount will be administered orally at t=0h. The glass cup will be washed two times with about 25 ml distilled water, gently swirreled and also orally administered. Two aliquots (2 ml) of the stock solution will pipetted in labelled sample tubes and stored in a freezer at ≤-18°C awaiting transportation and analytical determination of the dimethoate concentration. In addition a diluted sample (500 µl in 25 ml) will be prepared and two aliquots (2 ml) of the homogenous solution will be pipetted in labelled sample tubes and stored in a freezer at ≤-18°C awaiting transportation and analytical determination of the dimethoate concentration. The preparation of the study treatment solution will be carried out by the principal investigator or under his responsibility by qualified personnel.

8.9 Chemical and enzymatical analysis

All hemolysed blood samples will be analysed enzymatically for plasma and RBC cholinesterase according to a validated method (modified Ellman method) described by Meuling et al 1992 [2]. In brief: blood (40 µl) will be collected using heparinised calibrated capillaries after puncture of the finger-tip with a lancet. The capillary will be added to a tube containing containing 1 ml of a saponin solution (1 mg/ml) and shaken to complete hemolysis. Both plasma and RBC cholinesterase activities present will be determined with a modified colorimetric Ellman method using specific substrates (plasma; s-butyrylthiocholine and RBC; acetyl(β-methyl)thiocholine). In

each analysis run quality control samples containing both known cholinesterase activities will be co-analyzed. The dosing solution will be analysed for dimethoate only.
All urine samples will be analysed for dimethoate, omethoate, DCA, DTP, and TP according to validated LC-MS methods. In all urine (in study) samples the creatinin content of urine will be analyzed according to a standardised enzymatic method by the clinical chemistry department of TNO
The development and validation of the analytical methods (metabolite measurement in urine, dosing solution) will be done under the responsibility of the sponsor. Urine and dosing samples will be dispatched to and analysed by (except for creatinin) a GLP certified laboratory. Audited results (report) will be forwarded by the sponsor to TNO promptly after completion for inclusion in the (draft) final report.

8.10 Accountability

The person responsible for the application and administration of the test dilutions will keep an inventory, which includes a description of the formulation and the quantity of the investigational materials received for the study and a record of the materials that are dispensed and to whom and when they are dispensed.

9 Subjects

9.1 Population base

The subjects in this study will be recruited from the pool of volunteers of TNO Nutrition and Food Research. In case of an insufficient response, an advertisement will be applied in one of the local newspapers. However, the sponsor will be notified thereon in advance

About 20-25 possible candidates will be invited by letter for an oral information meeting at TNO. About 12-15 candidates will be subjected to the pre-study screen (see § 12.2.6). Based on the results of the pre-study screen 10-12 candidates will be subjected to the pre-selection phase (see § 12.2.7). Eligibility will be assessed based on these results and finally 8 (6+2 substitutes) subjects will be included in the study.

9.2 Number of subjects

Six (6) apparently healthy males will participate in the study.

9.3 Rationale for the number of subjects

The motivation of the number of 6 subjects for the treatment is based on experience and on the minimal number of subjects needed to calculate arithmetic means and standard deviations including (limited) insight in the inter-individual variation. The strategy of sampling and the number of samples has been selected to provide information on the urinary excretion profile of dimethoate and/or its metabolites and the possible effect on plasma and RBC cholinesterase activities, following a single oral administration of dimethoate.

9.4 Replacement

Subjects will only be replaced upon withdrawal before Day 01 of the study until the start of the experiment which is the actual oral administration of test substance. Subjects not completing the study for a non-treatment-related reason, will be considered non-completers. Subjects not completing the study for any treatment-related reason, will be considered treatment-related drop-outs.

9.5 Inclusion criteria

1. Age ≥18 years ≤ 45 years on Day 01
2. BMI >22 - <28 kg/m^2
3. Healthy as assessed by health questionnaire, physical examination and clinical chemistry (fasting state[1]) including: Haematology (RBC, WBC, platelets, Ht, Hb, Retics) and WBC blood differentiation; Clinical chemistry profile (ALT, ALP, AST, γ-GT, albumine, total bilirubin, creatinin, glucose and urea); Drug screen in urine (methadon, benzodiapines, cocaine, amphetamine/methamphetamine, opiaten, tetrahydrocannabiol, barbituraten, tricyclische antidepressiva); Dipstick urinalysis (protein, glucose, leucocytes, erythrocytes, nitrite, pH, ketones, urobilinogen, bilirubin); if the dipstick test gives values above the normal range for leucocytes, blood or protein, a microscopic inspection of urine sediment will be done.
4. Having given their written informed consent
5. Willing to comply with the study procedures
6. Voluntary participation
7. Willing to refrain from blood donation 30 days in advance of Day 01 and during the whole study
8. Willing to accept use of all anonymized data, including publication, and the confidential use and storage of all data
9. Willing to accept the disclosure of the financial benefit of participation in the study to the authorities concerned
10. Willing to refrain from intake of nutritional supplements during a period of 2 weeks prior to the study and ending at 72 h after the oral administration in the study

9.6 Exclusion criteria

Subjects with one or more of the following characteristics will be excluded from participation:

1. Participation in any clinical trial including blood sampling and/or administration of substances up to 90 days before Day 01 of this study
2. Under medical treatment of a specialist in the past and present for a reason that may affect the study outcome

[1]fasting state: no food, no drinks (other then water) from 22:00h the night before until sampling the next day

3. Use of prescribed medication (paracetamol excluded)
4. Alcohol consumption more than 28 units/week; (1 unit of alcohol equals 10 grams of ethanol)
5. Occupational exposure to, contact with, or use of agrochemicals
6. Physical training exceeding 10 hours of heavy weekly training or planning to train for an event requiring extreme physical training like triathlon or marathon
7. High background levels of the urinary metabolites to be measured, in two blank urine sample collected within three weeks prior to Day 01 of the study
8. Large variation (C.V. >15%)of plasma and RBC cholinesterase activities of three blank samples collected within three weeks prior to Day 01 of the study
9. TNO personnel and their relatives in the first and second remove
10. Having unsuitable veins for blood sampling
11. Not having a general practitioner
12. Not willing to accept information-transfer concerning participation in the study concerning participation in the study, or information regarding his health, like laboratory results, findings at anamnesis or physical examination and eventual adverse events to and from his general practitioner or his objection concerning participation
13. Not willing to accept transfer of medical information to and from the subjects' general practitioner when results of the study urgently demand for it, like laboratory results, findings at anamnesis or physical examination and eventual adverse events

10 Informing the subjects

The volunteers will be informed verbally on the aim, the study procedures, the constraints, insurance, and the confidentiality of the study and receive a copy of the written information for volunteers (P4802 B01). Those who wish to participate, will subsequently sign 2 copies of the informed consent form (P4802 F01) of which 1 copy they retain.

11 Study parameters

11.1 Kinetics

11.1.1 Individual and group urinary results

Individual concentration-time courses towards dimethoate, omethoate, DCA, DTP and TP in urine will be established in all subjects. These aforementioned concentration-time courses will also be established as corrected for the creatinin content of the urine sample.
The individual and group average urinary results will be expressed relative to the applied dose.

11.2 Dynamics

11.2.1 Individual cholinesterase results

Three individual pre-study plasma and RBC cholinesterase activities will be used to calculate the individual mean baseline level and the 95% confidence limits.
Individual in study plasma and RBC cholinesterase activities will be expressed relative to the mean pre-study individual cholinesterase baseline levels

11.3 Clinical laboratory tests (in study)

The following in study tests in the fasting state will be established:
Clinical chemistry: γ-GT, ALP, ALT, AST, total bilirubin, creatinin, urea, albumine, and glucose
Sampling scheme: pre-dose, and 24h post dosing
Dipstick urinalysis (protein, glucose, leucocytes, erythrocytes, nitrite, pH, ketones, urobilinogen, bilirubin; if the dipstick test gives values above the normal range for leucocytes, blood or protein, a microscopic inspection of urine sediment will be done
Sampling scheme: pre-dose
Haematology (RBC, WBC, platelets, Ht, Hb, Retics) and WBC blood differentiation
Sampling scheme: pre-dose

11.4 Additional tests

ECG recordings will be established four times during the clinical part of the study
Recording scheme: pre-dose, and at 3h, 24h and 72h post dosing
BP and HR will be established in study: pre-dose, 3h, 5h and 24h post dosing
Body weights will be established pre-dose.

11.5 Sample collection, codes and handling of samples

11.5.1 Blood collection (in study), handling and storage

Blood sampling (in study) will be performed by vene puncture pre-dose, and at predetermined time intervals in the study for the establishment of clinical laboratory tests (see § 12.3). Blood sampling (in study) will also be performed by finger-tip puncture using either lancets or an automated system pre-dose, and at pre-determined time intervals in study. All samples will be collected into heparinised calibrated capillaries (40μl). After sampling the capillary will be placed in a prelabelled tube containing 1 ml of a saponin solution (1 mg/ml). The tubes will be capped and shaken to hemolyse the blood. The hemolysates will be stored in a refrigerator at +2°C - 8°C awaiting transportation and determination of cholinesterase activities by SSDZ the same day. After the start of the oral administration sampling will performed up to about 72 hours post dosing.
Sampling scheme (vene puncture): pre-dose, and at 24h after the start of the oral administration (2 samples per subject; totally 12 samples)
Sampling scheme (hemolysates): pre-dose, 4h, 24h and 72h after the start of the oral administration (4 samples per subject; totally 24 samples)

11.5.2 Urine collection (in study), handling and storage

All produced urine (in study) will be collected from the onset of the oral administration in fractions up to about 72 hours post-dosing in containers according to pre-determined sampling scheme (see below). To establish baseline values prior to the start of the oral administration subjects will be asked to empty their bladder completely after rising and collect the urine sample (blank). Aliquots of 50-80 ml out of each urine fraction will be taken and transferred to a prelabelled container and about 1 ml to a prelabelled tube, the remainder will be discarded. All received samples will be stored in a freezer at ≤-18°C awaiting transportation either to the GLP certified laboratory or the clinical chemistry department of TNO.
Subjects will be asked to record the time of voiding onto the label of the particular container and to store the container at home in a cool and relatively dark place before transportation to TNO. Upon arrival at TNO recorded voiding times will be registered and the amount of urine will be determined by weighing and registered on P4802 F06.
Sampling scheme: overnight (blank), 0-4, 4-8, 8-12, 12-24, and 24-36, 36-48h and 48-72h after the onset of the oral administration (8 samples per subject; totally 48 samples).

11.5.3 Dosing solution

Two aliquots (2 ml) of the oral dosing solution (stock) and two aliquots (2 ml) of a prepared diluted sample (500 µl in 25 ml) will be pipetted in labelled sample tubes and stored in a freezer at ≤-18°C awaiting transportation and analytical determination of the dimethoate concentration.

11.6 Sample coding

Pre-screen serum/plasma and urine samples will be coded with preprinted labels as follows: Study code (4802), followed by a slash ('/'), followed by a pre-entry number (3 digits, starting at 101), followed by a slash ('/'), followed by a VTC[1] (3 digits).
Example: 4802/101/001

Post study screen serum/plasma and urine samples will be coded with preprinted labels as follows: Study code (4802), followed by a slash ('/'), followed by an entry number (2 digits), followed by a slash ('/'), followed by a VTC (3 digits).
Example:4802/01/003

In study serum/plasma and urine samples will be coded with preprinted labels as follows: Study code (4802), followed by a slash ('/'), followed by an entry number (2 digits), followed by a slash ('/'), followed by a VTC (3 digits). Example: 4802/01/005

Pre study collected urine samples will be coded with preprinted labels as follows: Study code (4802), followed by a slash ('/'), followed by an pre-entry number (3 digits), followed by a slash ('/'), followed by a matrix code letter and a sample sequence number (2 digits). Example: 4802/101/U01

[1]VTC = Visit Time Code

In study collected urine samples will be coded with preprinted labels as follows: Study code (4802), followed by a slash ('/'), followed by an entry number (2 digits), followed by a slash ('/'), followed by a matrix code letter and a sample sequence number (2 digits). Example: 4802/01/U01

Pre-selection collected hemolysed blood samples will be coded with preprinted labels as follows: Study code (4802), followed by a slash ('/'), followed by an pre-entry number (3 digits), followed by a slash ('/'), followed by a VTC (3 digits). Example: 4802/101/011

In study collected hemolysed blood samples will be coded with preprinted labels as follows: Study code (4802), followed by a slash ('/'), followed by an entry number (2 digits), followed by a slash ('/'), followed by a VTC (3 digits). Example: 4802/01/224

The remainder of the test substance (stock and dilution) to be used will be labelled with a preprinted label, containing the TNO study number (4802) followed by a matrix code letter and a sequence number (2 digits). Example: 4802/D01.

The following matrix codes will be used:

U for urine
D for dosing solution

The VTC-codes and matrix codes are listed in appendix P4802 B02

11.7 Remaining and archive samples

Remaining urine samples (pre-selection and in study) will be stored at ≤-18 °C at the GLP-certified laboratory for at least three months after date of the final report of the study.
All blood (hemolysed) and urine samples will be used only for measurement of plasma and RBC cholinesterase activities, and dimethoate, omethoate, DCA, DTP, TP and creatinin content, respectively, or for repeated analysis of these analytes.
After expiration of the above indicated storage time period the sponsor will be consulted and will in writing indicate the future of the stored material
All pre-screening blood, serum/plasma and urine samples will be used only for the measurements of safety parameters.
Remaining pre-study screening blood samples will be discarded after subject selection has been completed. Remaining post-screening blood and urine samples will be discarded after analysis

12 Study procedure

12.1 General

No examinations will be done before the volunteer concerned gave his written informed consent.

For both the pre-study period and the treatment periods checklists per volunteer will be used (P4802 forms F02 and F03, respectively).

The study consist of three (selection) phases: a) the in- and exclusion criteria, b) the pre-study screen and c) the pre-selection phase.

12.2 Pre-study screen, eligibility and selection

Candidates having informed TNO of being interested in possible study participation will be invited to come to TNO for an oral information session on the study

At the start of the oral information session, the "schriftelijke informatie voor proefpersonen" (P4802 B01; in Dutch) will be handed out. After oral information a booklet will be handed out containing the following forms:
1. Form P4802 F02 (subject checklist (pre-study))
2. Form P4802 F01 (written informed consent; in duplicate, in Dutch)
3. Form P4802 F04 (health and lifestyle questionnaire, in Dutch)
4. Form (physical examination)*
5. Form (eligibility checklist)
6. Form (participation history)
7 Form (end of trial)

*Forms refer to TNO Standard Operating Procedures (SOPs).

Candidates willing to participate will be asked, at first, to undersign both informed consent forms, thereafter to complete the health and lifestyle questionnaire. Candidates will receive one of the signed (volunteer and TNO) informed consent forms. Subsequently, each candidate will be subjected to a pre-study screen (see also § 12.2.6)
Based on the results, candidates will be subjected to a pre-selection phase, which includes (blank) cholinesterase activity measurements (3 times) and collection of two blank 24 h urine samples (see also § 12.2.7) Out of these group 6 subjects for inclusion in the study will be selected by drawing lots whereby 2 subjects will be assigned as substitute

Each subject will be allocated to a pre-entry number consisting of the TNO study code (4802), followed by a slash ('/'), followed by a 3-digit number starting at 101. The medical investigator will inform in writing the general practitioner of each volunteer who has signed the informed consent form on his application for study participation.

12.2.1 Medical history
Medical history will be assessed by the medical investigator on basis of the filled-in health and lifestyle questionnaire (Appendix P4802 F04).

12.2.2 Physical examination
Physical examination will be carried out by the (assistant) medical investigator

according to written instructions In addition the accessibility of veins will be investigated. Results will be recorded on form 'physical examination'.

12.2.3 ECG recording

ECGs will be conducted according to a TNO standard operating procedure and recorded with a 12-lead apparatus (Hewlett-Packard Pagewriter Xli). A reference value for inclusion, as judged by the medical investigator, will be <120ms. In case of any doubt an expert (cardiologist) will be consulted.

12.2.4 Vital signs

Systolic and diastolic blood pressure (BP) and heart rate (HR) will be measured oscillo-metrically in a sitting position and in the right arm after 1 min rest and recorded in mmHg and beats/min, respectively. Reference values for inclusion, as judged by the medical investigator, will be BP <90 (dia) and <160 (sys) and HR < 90 bpm.

12.2.5 Additional tests

Body weight will be measured the subject wearing indoor clothing, without shoes wallet and keys. Height will be measured without shoes. The BMI will be calculated.

12.2.6 Pre-study screen

The subjects will have a pre-study screening before the start of the treatment period. The pre-study screening will involve:

1. An interview (anamnesis) with the (assistant) medical investigator on basis of the completed health and lifestyle questionnaire
2. A physical examination and a visual inspection on the accessibility of the veins and recording of vital signs
3. Clinical laboratory tests (fasting state) will be performed including:
   Haematology (RBC, WBC, platelets, Ht, Hb, Retics) and WBC blood differentiation.
   Clinical chemistry profile (γ-GT, ALP, ALT, AST, total bilirubin, creatinin, urea, albumine, glucose).
   Drug screen in urine:methadon, benzodiapines, cocaine, opiaten, barbituraten, amphetamine/methamphetamine, tetrahydrocannabiol, tricyclische antidepressiva.
   Dipstick urinalysis (protein, glucose, leucocytes, erythrocytes, nitrite, pH, ketones, urobilinogen, bilirubin; if the dipstick test gives values above the normal range for leucocytes, blood or protein, a microscopic inspection of urine sediment will be done.

The methods used and the applicable reference values are given in P4802 B05 and P4802 B06, respectively

Results will be reported to the medical investigator for evaluation A copy of the laboratory report will be sent to the principal investigator
In case of clinically abnormal laboratory results, the medical investigator may decide to withdraw the subject and advise the subject's general practitioner.

12.2.7 Pre-selection of subjects

To investigate whether subjects urine comprises possible substances that may affect the analysis of the expected metabolites, two blank urine samples collected over 2 times 24 hours will be taken at least within three weeks prior to the actual administration (Day 01). In case a positive result occurs, subjects will be excluded from participation.

Also the individual baseline levels of plasma and RBC cholinesterase activities will be established. Therefore, three blank blood samples will be collected at least within three weeks prior to Day 01 of the study. From the analysis results the individual mean and 95%-confidence limits will be calculated. In case a large variation (C.V. >15%) in activities occurs, subjects will be excluded from participation.

12.2.8 Eligibility

Based on the results of the pre-selection, in consultation with the principal investigator and the sponsor, and the pre-study screening results, the medical - investigator will establish the eligibility.

The medical investigator will inform the subjects whether they are eligible or not and whether they will be invited to participate in the study or whether they are a substitute.

12.3 Inclusion and allocation to entry number

Subjects will be included in the study upon arrival on Day 01 at TNO. They all will be allocated, in order of arrival on Day 01, to a 2-digit entry number starting at 01.

12.4 Adverse events (AEs)

AEs will be established by the medical investigator on basis of:

1. Answer to the open question: 'How are you feeling?'
2. Spontaneous reporting
3. Well-being questionnaire (Form P4802 F05)

AEs will be classified by the medical investigator according to ICD-10, published by the WHO Collaborating Centre for International drug Monitoring, see Appendix P4802 B03. She will register her findings, conclusions and actions on standardized forms.

12.5 Serious Adverse Events (SAEs)

Any **serious** adverse event, whether or not related to the study treatment will be reported by the medical investigator immediately (within 24 hours) to the principal investigator, the sponsor or its representatives, the TNO Medical Ethics Committee (TNO-METC), the management of the TNO Target Organ Toxicology Department and to the subjects physician. A written report on the event will be sent to the sponsor and the TNO-METC within three working days.

See P4802 B03 for a definition of a serious adverse event.

12.6 Safety

The medical investigator will guard the medical safety of the subjects. During the pre-selection and the oral administration the medical investigator will be in the research facility. A state registered nurse or registered first-aider will be present in the research facility on all study days and when study visits have been scheduled.

12.7 Well-being

Well-being will be checked at the start and on study days 01, 02 and 04 at visits to TNO. The well-being will involve:

1. Filling-out a brief questionnaire (P4802 F05; in Dutch) at the beginning of the study Day 01, on Day 02 and Day 04;
2. Answer giving to the open question: 'How are you feeling?' at the end of study Day 01, on Day 02 and at the end of the clinical part (Day 04) of the study.

The answer to the open question will be recorded by the principal investigator or the research nurse on form P4802 F03.
At the end of the clinical part of the study all subjects will have a post-study screen.

12.8 Post study screen

The subjects will have a post-study screening at the end of the clinical part (Day 04).
The post-study screening will involve:

1. A (possible) exit-interview with the medical investigator initiated either by the medical investigator on reported AEs or complaints, or/and at the request of the subject.
2. Clinical laboratory tests (fasting state) will be performed including:
   Haematology (RBC, WBC, platelets, Ht, Hb, Retics) and WBC blood differentiation.
   Clinical chemistry profile (γ-GT, ALP, ALT, AST, total bilirubin, creatinin, urea, albumine, glucose). Dipstick urinalysis (protein, glucose, leucocytes, erythrocytes, nitrite, pH, ketones, urobilinogen, bilirubin; if the dipstick test gives values above the normal range for leucocytes, blood or protein, a microscopic inspection of urine sediment will be done.

On basis of the post study screen results, possible reported adverse events, and/or the exit interview the medical investigator will initiate further medical treatment as she deems useful.

12.9 Criteria for withdrawal or premature discontinuation

Subjects may discontinue the trial at any moment without the obligation to state the reason for discontinuation.
Subjects may be withdrawn from the study by the principal investigator if they do not comply with the rules and regulations of the study.
Subjects may be withdrawn from the study by the medical investigator in case of

reported serious adverse events or in case of other medical/social/psychological events as evaluated by the medical investigator and discussed with the principal investigator. Each subject who does not complete the study for any reason should have a post-study screen, if possible, at the end of the particular study day.

13 Documentation

The documentation of this study consists of the study protocol, correspondence, report, raw data, source documents or authenticated copies of these.
For privacy reasons, documents containing data of individual subjects will be identified only by their pre-entry or entry number

14 Statistics

14.1 Statistical analysis

Arithmetic means and standard deviations of the study parameters will be calculated. Additional parameters may be calculated if possible. Anthropometric data of the subjects will be presented descriptively

15 Reporting

The CRO will draft a report (in English), including a Summary, Introduction, Methods section, Description of Results and a Discussion section. The report will include deviations from the protocol and safety data. Results will be presented in summarizing tables. Individual results will be presented in appendices. The audited report from the GLP-certified laboratory will be presented in an appendix.
The final report will contain an audit certificate by the Quality Assurance Unit (QAU) of TNO Nutrition and Food Research and a statement on GCP compliance signed by the principal investigator.

16 Publication

Publication policy will be determined in mutual agreement between the sponsor and TNO

17 Timing

Approval of the METC-TNO has been obtained in Week 12 of 2003, the planned time schedule for the study will be as follows:

- Week 23, 2003 : Start recruitment of subjects
- Week 28-29, 2003 : Selection/screening/pre-selection
- Week 31, 2003 : Eligibility of subjects

- Week 32, 2003 : Study initiation clinical part
- Week 32, 2003 : Termination of clinical part
- Week 42, 2003 : Receipt audited chemical analysis results
- Week 44, 2003 : (Draft) final report (unaudited)
- Week≤52, 2003 : Audited final report

The actual start and termination dates of the study will be recorded in the final report

18 Insurance of subjects

At the request of DTF (principal, ="verrichter" (Dutch), TNO has insured the volunteers for damage or death caused by their participation (the pre-study selection included) in this study. The insurance company is:

Name	: Winterthur Schadeverzekering Maatschappij
Adress	: Postbus 83000, 1080 AA Amsterdam
Phone	: +31 20 5411754
Fax	: +31 20 6428428
Policy nr	: V00615407

The insurance covers damage with the following limits of indemnity (maxima): € 453,780.- per insured subject; € 6,806,703.- for the whole study and € 9,075,605.- as a maximum per annum

Insurance conditions will be presented in the written information (Appendix P4802 B01) for volunteers.

The Dutch law will prevail in case of legal dispute with a volunteer.

The volunteers will be informed how to handle in case of damage in the written information for volunteers (Appendix P4802 B01).

19 Ethics and quality

19.1 General

The study will only be conducted after written approval of the METC-TNO. The study will be conducted in compliance with the protocol and all (possible) amendments to the protocol. All amendments to the protocol effecting the design, rationale or objectives of the study, or the burden of or health risks for the volunteers will only be implemented after written approval of the METC-TNO and the sponsor. All amendments must be sent to the Medical Ethics Committee for information or approval.
The study will be conducted according to:

1. The current revision of the World Medical Association Declaration of Helsinki

(52nd Assembly, Edinborough, Scotland, October 2000).
2. The ICH Guidelines for Good Clinical Practice (ICH Topic E6, adopted 01-05-1996 and implemented 17-01-1997)
3. Regulations on Medical Research involving Human Subjects (Medical Research in Human Subjects Act; Wet medisch-wetenschappelijk onderzoek met mensen, WMO, 01-12-1999)
4. The current national regulations.

19.2 Good Clinical Practice (GCP)

TNO Nutrition and Food Research has been inspected by the Public Health Inspectorate for compliance with the principles of Good Clinical Practice for the conduct of clinical trials according to the ICH Guidelines for Good Clinical Practice (CPMC)/ICH/135/95). A written statement is supplied in P4802 B05.
The enzymatic analysis (cholinesterases) in hemolysed blood will be conducted by the Stichting Samenwerkende Delftse Ziekenhuizen (SSDZ).
The analytical determinations in urine samples and dosing solution will be conducted under responsibility of the sponsor by the selected GLP certified laboratory (Huntingdon Life Sciences Ltd).

19.3 Quality assurance and monitoring

The Quality Assurance Unit (QAU) of TNO Nutrition and Food Research will conduct audits during the study and will review the final report and the study files as required by the ICH Guideline for Good Clinical Practice (GCP). The audit certificate of the QAU will specify the dates of audits and reports to management and to the principal investigator.
To assure the quality of the cholinesterase measurements, an agreement will be made with the responsible laboratory (SSDZ) including a description of the methods, materials and apparatus used, and the use of control samples for the analytical method. The facility involved may be audited by the QAU of TNO Nutrition and Food Research.
The quality of the urine (metabolites) and dosing solution analysis carried out under the responsibility of the sponsor by a GLP certified laboratory (Huntingdon Life Sciences Ltd), will not be audited by the Quality Assurance Unit (QAU) of TNO Nutrition and Food Research.
An authenticated copy of the GLP certificate will be forwarded to TNO for archiving.

Members of the METC-TNO, representatives of the sponsor or regulatory authorities may conduct audits according to ICH Guideline for GCP of the testing facility and/or the raw data and will have direct access to medical records of subjects as produced and filed by the TNO medical investigator. To prevent interference with the study procedures, appointments for visits are requested in advance.

20 Retention of records, samples and specimens

The following documents will be retained in the archives of the TNO Nutrition and Food Research during 15 years after termination of the clinical phase of the study:

1. Master copies of the approved study protocol and final report
2. All documents containing personal data of individual trial subjects
3. Raw data (source documents or authenticated copies of these)
4. Correspondence
5. All other information related to tests and analyses conducted

All documents, raw data, correspondence and other study related information regarding the cholinesterase activity measurements present at SSDZ will be handed over to TNO for final archiving

All documents, raw data, correspondence and other study related information regarding the analytical determination of dimethoate (dosing solution) and urinary metabolites at Huntingdon Life Sciences Ltd, will be handed over to TNO for final archiving.

The following samples and specimens will be retained in appropriate facilities of TNO Nutrition and Food Research

1. A representative part of the test substance will be retained for 5 years if its nature allows it.
2. The remainder of the pre-selection and in-study urine samples and dosing solutions (stock and dilution) will be retained for at least three months after the final report has been approved by the sponsor.

After expiration of the above indicated storage time period the sponsor will be consulted and will in writing indicate the future of the stored material

21 References

1 Summary of mammalian toxicology and overall evaluation W. Köhl and M. Hofer. SCC document 104-084, 15 November 2002.

2. Meuling,W.J.A., M.J.M. Jongen and J.J. van Hemmen, An automated method for the determination of acetyl and pseudo cholinesterase in hemolysed whole blood, 1992, Am. J. Ind. Med, 22, 231-241

22 Approval of the protocol

22.1 Sponsor

P. Hofmann
Responsible person for DTF

see next page
Signature

- -
Date (dd-mm-yy)

22.2 TNO Nutrition and Food Research

W.J.A. Meuling, BSc.
Principal Investigator

Signature

15-05-03
Date (dd-mm-yy)

W.A.A. Klöpping-Ketelaars, PhD, MD
Medical Investigator

Signature

16-05-03
Date (dd-mm-yy)

H. Bekenes
Co-investigator (SSDZ)

Signature

- -
Date (dd-mm-yy)

H.Harper
Co-investigator (Huntingdon)

see next page
Signature

- -
Date (dd-mm-yy)

A.F.M. Kardinaal, PhD, MSc
Head, Department of
Nutritional Physiology

Signature

21-05-03
Date (dd-mm-yy)

22 Approval of the protocol

22.1 Sponsor

P. Hofmann
Responsible person for DTF

Signature 4.6.03
Date (dd-mm-yy)

22.2 TNO Nutrition and Food Research

W.J.A. Meuling, BSc
Principal Investigator

Signature 15-05-03
Date (dd-mm-yy)

W.A.A. Klöpping-Ketelaars, PhD, MD
Medical Investigator

Signature 16-05-03
Date (dd-mm-yy)

H. Bekenes
Co-investigator (SSDZ)

see next page
Signature
Date (dd-mm-yy)

H Harper
Co-investigator (Huntingdon)

Signature 02-06-03
Date (dd-mm-yy)

A.F.M. Kardinaal, PhD, MSc
Head, Department of
Nutritional Physiology

Signature 21-05-03
Date (dd-mm-yy)

22 Approval of the protocol

22.1 Sponsor

P. Hofmann
Responsible person for DTF — Signature — Date (dd-mm-yy)

22.2 TNO Nutrition and Food Research

W.J.A. Meuling, BSc.
Principal Investigator — Signature — Date (dd-mm-yy): 15-05-03

W.A.A. Klöpping-Ketelaars, PhD, MD
Medical Investigator — Signature — Date (dd-mm-yy): 16-05-03

H. Bekenes
Co-investigator (SSDZ) — Signature — Date (dd-mm-yy): 22_05_03

H.Harper
Co-investigator (Huntingdon) — Signature — Date (dd-mm-yy)

A.F.M. Kardinaal, PhD, MSc
Head, Department of
Nutritional Physiology — Signature — Date (dd-mm-yy): 21-05-03

23 List of Appendices

P4802 B01	:	Written information for volunteers (in Dutch and English)
P4802 B02	:	Visit time and matrix codes
P4802 B03	:	Adverse Events: definitions and codes
P4802 B04	:	Statement of GCP compliance
P4802 B05	:	Methods of clinical laboratory tests
P4802 B06	:	Reference values of clinical laboratory tests
P4802 B07	:	Distribution list
P4802 B08	:	Information on dimethoate

24 List of forms

P4802 F01	:	Written informed consent (in Dutch and English)
P4802 F02	:	Subjects checklist (pre-study)
P4802 F03	:	Subjects checklist (in study)
P4802 F04	:	Health and lifestyle questionnaire (in Dutch and English)
P4802 F05	:	Well-being questionnaire (in Dutch and English)
P4802 F06	:	Quantitative urine collection, sub-sample preparation and sample transfer to chemical laboratory (pre-selection and in study)
P4802 F07	:	Transfer of dosing samples to chemical laboratory
P4802 F08	:	Blood collection, hemolysate preparation and sample transfer to clinical chemical laboratory (preselection and in study)
P4802 F09	:	Blood and urine collection and sample transfer to clinical laboratory (pre-study)
P4802 F10	:	Blood and urine collection and sample transfer to clinical laboratory (in- study and post-study)

Korte titel onderzoek: **Orale absorptie van dimethoaat**
Titel bijlage: **Schriftelijke informatie voor vrijwilligers**

Titel: **Uitscheiding via de urine van dimethoaat en omzettingsproducten na een eenmalige orale dimethoaat toediening in mannelijke vrijwilligers**

Onderzoeksleider: Ing. W.J.A Meuling
Arts: Dr. W.A.A. Klöpping-Ketelaars, arts
Hoofdverpleegkundige: F.W. Sieling

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Arts buiten kantooruren: 06 22805921
06 22805922

VERTROUWELIJKHEID

INHOUDSOPGAVE **pagina**

1 INLEIDING

1.1 TNO

TNO staat voor Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek en is opgericht in 1932. TNO maakt de resultaten van natuurwetenschappelijk onderzoek toepasbaar voor de samenleving. TNO bestaat uit diverse instituten, waarvan TNO Voeding er één is

1.2 Onderzoek bij TNO Voeding

TNO Voeding' verricht onderzoek naar de gunstige en ongunstige aspecten van voeding, naar de samenstelling, verwerking en bereiding van voedingsmiddelen en diervoeding, naar de werking van geneesmiddelen, maar ook naar de gewenste en ongewenste effecten van cosmetische en antiseptische producten en naar de effecten van (industriële) chemische stoffen op de gezondheid van de mens.

2 REDEN VAN HET ONDERZOEK

In de agrarische sector worden o.a. insecticiden (gewasbeschermingmiddelen) regelmatig gebruikt. Dimethoaat is zo'n insecticide dat wordt gebruikt op allerlei planten, fruit, groeten, etc. om bescherming te bieden tegen een groot aantal plagen. Een veldonderzoek waarbij de mate van blootstelling aan dimethoaat van werknemers in de agrarische sector zal worden onderzocht is in voorbereiding. Hiervoor is het noodzakelijk om over gegevens te beschikken hoe en hoe snel deze stof via de urine wordt uitgescheiden, hetgeen in deze studie wordt onderzocht. De experimentele condities in deze studie zijn zo gekozen dat het geenszins te verwachten is dat er een gezondheidsrisico voor de vrijwilligers door hun deelname aan het onderzoek zal optreden.

3 DOEL VAN HET ONDERZOEK

Dit onderzoek is bedoeld om inzicht te krijgen in het gedrag van de stof in het lichaam (metabolisme) en het uitscheidingsprofiel in de urine van dimethoaat en de door het lichaam gevormde omzettingsprodukten van deze stof.

4 OPDRACHTGEVER

Dit onderzoek wordt uitgevoerd in opdracht van de 'Dimethoate Task Force' te Duitsland

5 PROEFOPZET

Aan het onderzoek kunnen 6 mannen met een leeftijd vanaf 18 jaar t/m 45 jaar meedoen. De onderzoeksperiode omvat 4 dagen, in welke periode men alle geproduceerde urine moet verzamelen en op 3 dagen naar TNO dient te komen voor bloedafname. Op de eerste onderzoeksdag vindt de toediening van de onderzoeksstof dimethoaat plaats. De stof zal zijn opgelost in een kleine hoeveelheid water, dat moet worden opgedronken. Voorafgaand aan het eigenlijke onderzoek moet u viermaal naar het onderzoeksinstituut te komen voor een keuring en een voorselectie waarbij afname van een druppel bloed (vingerprik) op 3 verschillende dagen zal plaatsvinden. Tevens moet u gedurende 2 perioden van 24 uur uur alle urine verzamelen.
De urine zal worden geanalyseerd op de aanwezigheid van de onderzoeksstof en omzettingsproducten. Het bloed zal worden gebruikt voor een enzym activiteits bepaling (cholinesterase).

6 SELECTIE

Voordat u mag deelnemen aan het onderzoek vindt er een selectie plaats. Uw deelname aan het onderzoek hangt af van de uitslag van de selectie. De selectie bestaat uit de volgende stappen:

1. U krijgt mondelinge en schriftelijke informatie over het onderzoek
2. U wordt gevraagd het formulier 'toestemming deelneming' te ondertekenen
3. U wordt gevraagd een vragenlijst over uw gezondheid en leefgewoonten in te vullen
4. U wordt gekeurd aan de hand van de lijst met toelatings- en uitsluitingscritria (zie bijlage 1). Deze keuring bestaat uit een gesprek met de arts over de ingevulde vragenlijst, uw lengte en gewicht worden bepaald, er wordt bloed en urine onderzocht, uit veiligheidsoverwegingen wordt uw bloeddruk, hartslag en een elektocardiogram (ECG; hartfilmpje) gemaakt en er wordt een algemeen lichamelijk onderzoek gedaan. Indien u geschikt bent voor het onderzoek volgens het gezondheidskundig onderzoek, vindt er een verdere selectie plaats (pre-selectie) op basis van analyse van het enzym cholinesterase in 3 afgenomen bloedmonsters (vingerprik) en de aanwezigheid van de onderzoeksstof en omzettingsproducten in verzamelde urine gedurende twee 24-uurs perioden.
5. U hoort of u bent goedgekeurd voor het onderzoek en indien niet waarom niet. Als u bent goedgekeurd hoort u of u bent geselecteerd voor het onderzoek of dat u reserve bent, of bent uitgeloot

7 UITVOERING VAN HET ONDERZOEK (alleen geselecteerde deelnemers)

7.1 Tijdsplanning en duur van het onderzoek

Het programma van het onderzoek kunt u vinden in bijlage 2.
Het onderzoek vindt plaats in het onderzoekscentrum van TNO Voeding te Zeist

Het onderzoek beslaat een tijdsperiode van maandag **4 augustus** tot en met donderdag **7 augustus 2003**. Op maandag 4 augustus dient u nuchter om 8.00 uur bij het onderzoekscentrum van TNO Voeding te aanwezig te zijn voor de toediening van de onderzoeksstof en afnemen van bloed. Na de

lunch kunt u TNO verlaten. De volgende dag en drie dagen na de toediening wordt u weer nuchter bij TNO verwacht voor de afname van een buisje bloed, een vingerprik en het inleveren van de (thuis) verzamelde urine. Op elke dag wordt ook een ECG gemaakt
Tijdens uw verblijf bij TNO op de eerste onderzoeksdag bestaat er de mogelijkheid, na de toediening om u te amuseren door b.v. TV te kijken, computerspelletjes te doen, te lezen of te studeren. Op deze dag zal u worden voorzien van ontbijt (broodjes) en een lunch en zal er koffie en thee in voldoende mate aanwezig zijn.

7.2 Onderzoeksproduct en hoeveelheid

Het onderzoek wordt uitgevoerd met het insecticide (gewasbeschermingsmiddel) dimethoaat dat in hoge concentraties giftig is. Voor het onderzoek wordt een kleine hoeveelheid dimethoaat in gedestilleerd water opgelost. De gekozen hoeveelheid van deze stof is gelijk aan de zogenaamde 'acute referentie dosis' (= 0.03 mg/kg lichaamsgewicht), welke als volgt is tot stand gekomen. Via een onderzoek met proefpersonen gedurende 39 dagen is een 'no effect level' (dosis waarbij de gevoeligste parameter niet veranderd) vastgesteld welke overeen kwam met een dagelijkse hoeveelheid van 0.3 mg/kg lichaamsgewicht. Om te compenseren voor eventuele tussenpersoons verschillen in gevoeligheid wordt hierbij een veiligheidsfactor van een factor 10 gehanteerd.

7.3 Bloed en urine analyses

In deze studie zullen bloedmonsters worden onderzocht op de activiteit van twee enzymen nl. plasma- en RBC (rode bloed cel)-cholinesterase. Deze bloedmonsters zullen worden uitgevoerd door en hiervoor verstuurd aan een groot klinisch-chemisch laboratorium te Delft; (SSDZ) Stichting Samenwerkende Delftse Ziekenhuizen. Urinemonsters zullen worden onderzocht op het gehalte aan dimethoaat, aan dimethoaat afbraakprodukten en creatinine. Deze analyses zullen worden uitgevoerd onder de verantwoordelijkheid van de opdrachtgever in een daartoe gekwalificeerd engels laboratorium (Huntingdon Life Sciences Ltd.). De urinemonsters zullen hiervoor naar dit laboratorium worden opgestuurd. Het creatininegehalte van de urinemonsters zal worden onderzocht bij TNO.
Alle bloed en urinemonsters worden alleen op de hierboven aangegeven stoffen onderzocht.

7.4 Veiligheid en mogelijke bijwerkingen

Vanwege de lage dosis dimethoaat die in deze studie wordt toegediend wordt geen enkele bijwerking verwacht. De algemene veiligheids-procedures voor klinisch onderzoek bij TNO zullen verder worden gevolgd. Bij elk bezoek aan TNO vult u een formulier in met vragen over uw gezondheid sinds het vorige bezoek. De arts beoordeelt deze vragenlijst en neemt zo nodig contact met u op. Tijdens alle bezoeken is een verpleegkundige of een gediplomeerde EHBO-er aanwezig.

8 UITKEURING

Voor het vertrek bij TNO aan het einde van elke onderzoeksdag wordt door een medewerker van TNO Voeding geïnformeerd naar het geestelijk en lichamelijk welbevinden van de vrijwilliger. Een

uitkeuringinterview met de behandelend arts vindt plaats op verzoek van de arts, of op basis van geuitte klachten of op uw verzoek. Er zal een lichamelijkonderzoek plaatsvinden en urine en bloed worden afgenomen (2 buisjes) waarvan o.a. het bloedbeeld zal worden bekeken. Verder zal er een ECG (hartfilmpje) worden opgenomen en zullen bloeddruk en hartslag worden bepaald.

9 TE VERWACHTEN ONGEMAKKEN

Als u deelneemt aan het onderzoek kunt u (eventueel) de volgende ongemakken verwachten:

1. Lichamelijk onderzoek voor de inkeuring (beluisteren van de longen, het testen van reflexen, het bekloppen van de buik)
2. Het invullen van een vijftal vragenlijsten
3. Achtmaal (inclusief de selectiedagen) een bezoek (gewoonlijk 1 à 2 uur, éénmaal een hele ochtend) brengen aan het onderzoekscentrum van TNO
4. Het gedurende een periode van 2 x 24 uur en een periode van 72 uur na de toediening van de stof verzamelen van alle urine
5. Opname van een drietal ECGs (hartfilmpjes)
6. Het opdrinken van een glas water (25 ml) met de onderzoeksstof en het 2x naspoelen en opdrinken.
7. Het regelmatig afnemen van een druppel bloed: zevenmaal via een vingerprik op verschillende dagen.
8. Het viermaal afstaan van bloed (ca 10 ml per keer), te weten voor en na de studie voor de in- en uitkeuring en op twee momenten tijdens de onderzoeksperiode
9. Het nuchter moeten komen bij TNO voor de inkeuring en vervolgens op 3 studiedagen.
10. Het niet mogen gebruiken van voedingssupplementen, zoals vitaminepreparaten en extracten van planten, gedurende een periode van 2 weken, eindigend 3 dagen na de toediening van de onderzoeksstof.

10 ETHISCHE ASPECTEN, KWALITEIT EN PERSOONLIJKE LEVENSSFEER

10.1 Voorwaarden van het onderzoek

Het onderzoek wordt uitgevoerd volgens erkende richtlijnen en regels die u terug kunt vinden in bijlage 3.

10.2 Informatie en schriftelijke toestemming

U kunt pas deelnemen aan het onderzoek, nadat u mondeling en schriftelijk bent geïnformeerd over de achtergronden van de procedures tijdens het onderzoek, de mogelijke risico's bij deelname aan het onderzoek en uw rechten en plichten. Ook moeten al uw vragen naar tevredenheid zijn beantwoord en moet u schriftelijk toestemming hebben gegeven voor uw deelname ('informed consent'). Indien er nieuwe relevante informatie beschikbaar komt met betrekking tot het onderzoek zal u daarover worden geïnformeerd.

10.3 Beëindiging deelneming

U doet geheel vrijwillig mee aan het onderzoek. U mag uw deelname aan het onderzoek op ieder moment beëindigen, en u hoeft daarbij geen reden op te geven. De arts kan uw deelname aan het onderzoek beëindigen, als hij/zij dat op medische gronden noodzakelijk vindt. De onderzoeksleider kan uw deelname beëindigen, als u zich niet aan de voorschriften houdt. Bovendien kan TNO Voeding of de opdrachtgever besluiten het onderzoek te beëindigen.

10.4 Onafhankelijke arts

Als u twijfelt of u wel geschikt/gezond bent om aan dit onderzoek deel te nemen kunt u met uw vragen terecht bij de arts, onderzoeksleider of de hoofdverpleegkundige. Als u desondanks nog met vragen blijft zitten kunt u contact opnemen met een onafhankelijke arts, Dr. E.J. van der Beek. Hij is te bereiken op werkdagen via **070-3181887** of **06-51383487** (Arbo Management Groep, Postbus 82001, 2508 EA, Den Haag).
Ook als u als deelnemer aan dit onderzoek medische vragen heeft die u liever aan een onafhankelijke arts wilt stellen kunt u met uw vragen bij hem terecht.

10.5 Persoonlijke levenssfeer en toegankelijkheid onderzoeksgegevens

Om de privacy te waarborgen, wordt het gebruik van namen zo veel mogelijk beperkt. Iedere (kandidaat)vrijwilliger krijgt een keurings- en een onderzoeksnummer. Deze nummers worden gebruikt bij de registratie van de onderzoek-gegevens.
Tijdens het onderzoek kunnen daartoe bevoegde TNO-medewerkers, inspecteurs van de overheid en monitoren van de betrokken opdrachtgever ter plekke aanwezig zijn om inspecties en/of beoordelingen uit te voeren om de betrouwbaarheid en de kwaliteit van de meetgegevens te verifiëren waarop later de onderzoeksresultaten worden gebaseerd. De inzage in uw persoonlijke gegevens gebeurt steeds onder geheimhouding. Zie bijlage 4 voor meer informatie over de keurings- en onderzoeksnummers en over welke personen inzage mogen hebben in documenten met uw identiteit en meetgegevens.
De resultaten van het onderzoek kunnen leiden tot publikaties in vaktijdschriften en voordrachten op congressen en bijeenkomsten. Uw anonimiteit blijft daarbij gewaarborgd.
Uw huisarts zal door TNO worden geïnformeerd over uw deelname aan het onderzoek en als er afwijkende waarden zijn gevonden in het bloed of bij de andere metingen, worden u en hij/zij ook hierover geïnformeerd.

11 FINANCIËLE VERGOEDING

Voor deelname aan het volledige onderzoek, ontvangt u een vergoeding van **€ 300,-** (bruto). Indien u wilt deelnemen en gezondheidskundig wordt goedgekeurd, maar gedurende de voorselectie op grond van de bloed- en urine analyses niet wordt geselecteerd ontvangt u een vergoeding van **€ 75,-**. Reserves voor het onderzoek ontvangen daarnaast nog een vergoeding van € 50 (1e reserve) en € 25 (2e reserve).

TNO is verplicht deze vergoeding op te geven aan de belastingdienst. In bijlage 5 kunt u lezen welk deel van de vergoeding u ontvangt bij onvolledige deelname.

12 VERZEKERING

Tijdens het onderzoek bent u verzekerd tegen de gevolgen van letsel (= lichamelijke en geestelijke schade) veroorzaakt door:

1. Deelname aan het onderzoek en de voorafgaande informatie- en selectieprocedure. en
2. Gaan naar, verblijf bij en vertrek van TNO Voeding.

Zie bijlage 6 voor meer informatie over de verzekering.

Bijlage 1. Selectiecriteria

Toelatingscriteria

1 Mannen; leeftijd vanaf 18 tot en met 45 op dag 01 van het onderzoek
2 Body Mass Index (BMI): >22 en <30 kg/m^2 (=gewicht gedeeld door de lengte in meters in het kwadraat)
3. Geschikt op grond van medische anamnese, het lichamelijk onderzoek, en het bloed- en urineonderzoek. Naast het gebruikelijke bloed- en urineonderzoek zal ook onderzoek (urine) naar het eventueel gebruik van drugs plaatsvinden (methadon, benzodiapines, cocaine, opiaten amphetamine/methamphetamine, tetrahydrocannabiol, barbituraten, tricyclische anti-depressiva).
4 Schriftelijke toestemmingsverklaring na mondelinge en schriftelijke uitleg over aard en doel van het onderzoek
5 Vrijwillig deel te nemen
6 Bereid om gedurende 30 dagen voorafgaand aan studiedag 01 en tijdens de studie niet als bloed-donor te dienen
7. Bereid te accepteren dat de verzamelde gegevens anoniem worden gebruikt, gearchiveerd en mogelijk worden gepubliceerd
8. Bereid zich aan de regels van het onderzoek te houden
9 Bereid te accepteren, dat de ontvangen vergoeding door INO wordt opgegeven aan de belastingdienst
10 Bereid om af te zien van het gebruik van voedingssupplementen gedurende een periode van 2 weken, eindigend 3 dagen na de toediening van de onderzoeksstof.

Uitsluitingscriteria

1. Deel hebben genomen aan enig onderzoek met bloedafnames en/of toediening van producten in de 90 dagen voorafgaande aan studiedag 01 van dit onderzoek
2. Onder medische behandeling geweest of nog voortdurend voor een aandoening welke invloed zou kunnen hebben op de resultaten van het onderzoek
3. Gebruik van medicijnen anders dan paracetamol
4. Alcohol gebruik van meer dan 28 glazen per week
5. Beroepsmatige blootstelling aan, of in contact komend met, of gebruik van agrochemicaliën.
6. Het uitvoeren van zware sport trainingsarbeid (> 10 uur per week) of in training zijn voor zeer zware sportevenementen als een marathon of triathlon.
7. Hoge achtergrond concentraties van dimethoaat metabolieten of andere stoffen in (blanco) urine verzameld op twee dagen in een periode van drie weken voorafgaand aan dag 01
8. Grote mate van variatie (CV >15%) van de plasma en RBC cholinesterase activiteitniveaus gemeten in 3 bloeddruppels afgenomen in een periode van 2 weken voorafgaand aan dag 01
9. TNO-medewerkers of hun familieleden (tot en met de tweede graad)
10. Het hebben van aderen die ongeschikt zijn voor bloedafname
11 Het niet hebben van een huisarts
12. Het niet toestaan dat informatie aangaande deelname aan de studie wordt doorgegeven aan de eigen huisarts of dat, indien de resultaten van het onderzoek, zoals laboratorium uitslagen, bevindingen bij lichamelijk onderzoek, de anamnese en eventuele optredende bijwerkingen daartoe aanleiding geven voor wat betreft de gezondheid, contact wordt opgenomen met de eigen huisarts voor het doorgeven van en/of het inwinnen van medische gegevens en het bezwaar van de huisarts tegen deelname
13. Het niet toestaan dat, indien de resultaten van het onderzoek daartoe aanleiding geven voor wat betreft de gezondheid, contact wordt opgenomen met de eigen huisarts voor het doorgeven van en/of het inwinnen van medische gegevens

Bijlage 2. Overzicht van metingen tijdens het onderzoek

Nadat u geselecteerd bent voor het onderzoek wordt van u verwacht dat u gedurende de periode van 03 maart tot 06 maart 2003 TNO verscheidene keren bezoekt. Op deze bladzijde wordt aangegeven wanneer u tijdens het onderzoek naar TNO moeten komen, en welke handelingen daar worden verricht
[Bijgaande schema's met tijden zijn indicatief; de werkelijke tijden kunnen hiervan iets afwijken]

DAG 01 (Maandag)

08 00	Aankomst bij TNO (nuchter)
	Registratie, check 'Well-being'
	Mondelinge instruktie over het experiment
08:10	Bepaling lichaamsgewicht
08 15	Produceren van een urinemonster (blanco): blaas ledigen
	Afname van een druppel bloed (vingerprik) en afname van 2 buisjes bloed via de ader
08 20	koffie/thee + 2 broodjes
08 30	Opdrinken van het glaasje water met dimethoaat
	Koffie of thee
11 30	Afname van een druppel bloed (vingerprik)
11 35	Opname van een elektrocardiogram (ECG; hartfilmpje)
12 00	Instruktie (mondeling) voor de urinezameling thuis
12 30	Produceren van een urinemonster
± 13:00	Lunch
	Uitreiking urineverzamelflessen en verzamelschema
13:15	'How are you feeling"
13:30	Naar huis
	Thuis verzamelen van urine

Dag 02 (Dinsdag)

08 00	Aankomst bij TNO (nuchter), Check Well-being
	Inleveren van thuis verzamelde urine: 4-8 uur, 8-12 uur en 12-24 uur
08 30	Afname van een druppel bloed (vingerprik) en afname van een buisje bloed via de ader
08 35	Opname van een elektrocardiogram (ECG; hartfilmpje)
08 45	Koffie/thee + broodjes
08 55	Uitreiking urineverzamelflessen en verzamelschema
09 00	'How are you feeling"
09 05	Naar huis
	Thuis verzamelen van urine: 24-36 uur, 36-48 uur, 48-72 uur

Dag 03 (Woensdag)

Thuis verzamelen van urine

Dag 04 (Donderdag)

08.00	Aankomst bij TNO (nuchter), registratie en check Well-being
	Inleveren van van thuis verzamelde urine: 24-36 uur, 36-48 uur
08.30	Afname van een druppel bloed (vingerprik)
08.35	Uitkeuring
	Bloedafname (2 buisjes) + een beetje urine
08.40	Blaas ledigen in urinecontainer 48-72 uur
08.45	Thee/koffie + broodjes
08.50	Opname van een elektrocardiogram (ECG; hartfilmpje)
09.00	Mogelijk uitkeuringsinterview
09.20	Ondertekenen van uitbetalingsformulier
09:40	Vertrek van TNO

Bijlage 3. Erkende richtlijnen en regels

De erkende richtlijnen en regels volgens welke het onderzoek wordt uitgevoerd zijn:

1. Volgens de beginselen van de 'World Medical Association Declaration of Helsinki, laatstelijk gereviseerd tijdens de 52ste WMA General Assembly, Edinburgh, Schotland, October 2000.
2. Volgens de beginselen voor Good Clinical Practice (GCP), neergelegd in de zogenoemde richtlijnen van de ICH (International Conference on Harmonisation) voor klinisch onderzoek met proefpersonen (ref: ICH TOPIC E6, 01-05-1996)
3. Volgens de Wet medisch-wetenschappelijk onderzoek met mensen (WMO, 01-12-99)
4. Nadat een positief oordeel is verkregen van de Medisch-Ethische Toetsingscommissie INO.

Bijlage 4. Keurings- en onderzoeksnummers en personen die inzage mogen hebben in documenten met uw identiteit en meetgegevens

Het gebruik van namen wordt beperkt tot één sleutellijst met de combinatie naam en keuringsnummer en formulieren 'Toestemming deelneming' en 'Verklaring leefgewoonten en gezondheid' Deze documenten worden door TNO Voeding gearchiveerd.
Daarnaast worden namen gebruikt bij het per post versturen van informatie aan u of aan uw (huis)arts

De door TNO Voeding verzamelde documenten en onderzoeksgegevens worden tenminste 15 jaar gearchiveerd door TNO Voeding.

Documenten die uw identiteit vermelden, mogen niet tegelijk met documenten die meetgegevens bevatten, worden ingezien, behalve door:

1. De (plaatsvervangend) projectleider
2. De (plaatsvervangend) arts
3. De assistent arts
4. De hoofdverpleegkundige
5. Daartoe bevoegde kwaliteitsfunctionarissen van het Instituut TNO Voeding
6. Daartoe bevoegde inspecteurs van overheidsinstanties
7. Daartoe bevoegde monitoren en inspecteurs van de betrokken opdrachtgever
8. Leden van de Medisch Ethische Toetsingscommissie van TNO

Bovenstaande functionarissen zijn allen verplicht tot geheimhouding van uw persoonlijke gegevens. Indien u besluit deel te nemen aan dit onderzoek geeft u tevens toestemming voor inzage in die gegevens.

Bijlage 5. Financiële vergoeding bij onvolledige deelname

Bij onvolledige deelname aan het onderzoek ontvangt u:

1. De volledige vergoeding, als uw deelname wordt beëindigd door:
   1. De arts op medische gronden.
   2. De onderzoeksleider op basis van onderzoeksafwijkingen buiten uw schuld om (ter beoordeling van de projectleider)
   3. Voortijdige beëindiging van het onderzoek door de opdrachtgever of TNO Voeding.

2. Een pro rata vergoeding, als:
   1. U uw deelname voortijdig beëindigt op grond van overmacht (ter beoordeling van de projectleider).
   2. U reserve bent voor insluiting in het onderzoek.

3. Geen vergoeding, als:
   1. U zonder opgaaf van redenen voortijdig uw deelname aan het onderzoek beëindigt.
   2. U met opgaaf van reden, die echter geen overmacht betreft (ter beoordeling aan de projectleider), voortijdig uw deelname aan het onderzoek beëindigt.
   3. De projectleider voortijdig uw deelname beëindigt op grond van gebrek aan medewerking aan het onderzoek of wangedrag
   4. U niet geschikt bent voor insluiting in het onderzoek.
   5. U wel geschikt bent voor insluiting in het onderzoek, maar geen reserve bent.

De projectleider beslist in overige gevallen van voortijdige beëindiging van deelname aan het onderzoek of u een vergoeding ontvangt en, zo ja, welk bedrag

Bijlage 6. Aanvullende informatie over de verzekering

INO heeft als verrichter, conform het Besluit van de Minister van Justitie en de Minister van Volksgezondheid, Welzijn en Sport van 5 juli 1999, een risicoverzekering afgesloten voor proefpersonen. De verzekering ia afgesloten bij:

Winterthur Schadeverzekering Maatschappij
Postbus 83000
1080 AA Amsterdam
Tel (020) 5411754
Fax (020) 6428428
Polis nr V00615407

Onder de polis is schade als gevolg van deelname aan het onderzoek verzekerd.
Onder schade wordt verstaan schade door letsel of overlijden.
De verzekering biedt dekking voor schade tot een bedrag van € 453 780,- per proefpersoon, zulks tot een maximum van tot € 6.806.703,- voor het onderzoek in zijn geheel en gelimiteerd tot € 9.075.605,-- per verzekeringsjaar.

Uitgesloten van dekking is schade:

1 Waarvan nagenoeg zeker was dat deze zich bij de proefpersoon zou voordoen;
2. Die zich bij nakomelingen openbaart als gevolg van een nadelige inwerking van het onderzoek op genetisch materiaal van de proefpersoon;
3. Door aantasting van de gezondheid van de proefpersoon die zich ook zou hebben geopenbaard wanneer de proefpersoon niet aan dit onderzoek had deelgenomen;
4. Die het gevolg is van het niet, of foutief opvolgen van de aanwijzingen en instructies die u door TNO zijn of worden gegeven.

Bij letsel en letselschade moet(en):

1 contact worden opgenomen met de hoofdverpleegkunidige van TNO Voeding,
2. de adviezen van de onderzoeker van TNO Voeding worden opgevolgd,
3. er zorg voor worden gedragen dat verdere schade zo veel mogelijk wordt beperkt/voorkomen, en
4. de huisarts worden geïnformeerd.

Als u meent materiële schade te hebben ondervonden waarvoor TNO aansprakelijk is, neem hierover dan contact op met de hoofdverpleegkundige van TNO Voeding.
Schadeclaims worden na aanmelding afgehandeld door TNO.

Brief title of study: Oral absorption of dimethoate
Title of appendix: **Written information for volunteers**

Title: Urinary excretion profile of dimethoate and its metabolites after single oral administration of dimethoate in healthy male volunteers.

Principal investigator: W.J.A. Meuling, BSc
Medical investigator: W.A.A. Klöpping-Ketelaars, PhD. MD
Senior research nurse: F.W. Sieling

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outside ordinary business hours: +31 06 53943338

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(during business hours)

CONTENTS

1 INTRODUCTION

1.1 TNO

TNO stands for Netherlands Organization for Applied Scientific Research. TNO was established in 1932. TNO aims at making results of scientific research applicable for society. TNO consists of several institutes, including TNO Nutrition and Food Research.

1.2 Research at TNO Nutrition and Food Research

The institute TNO Nutrition and Food Research is engaged in research into favourable and adverse effects of food products, the composition, processing and preparation of foods and animal feeds, the action of pharmaceutical products (TNO Pharma), desirable and undesired effects of cosmetic and antiseptic products and the effects of (industrial) chemicals on human health.

2 STUDY RATIONALE

In agriculture chemicals are being used to protect or cure all kinds of plants, fruit and crops from diseases. Dimethoate is such an active chemical (insecticide) were workers may be exposed to. This study is undertaken to investigate the urinary excretion profile of dimethoate and/or its metabolites after a single oral administration of dimethoate. The results of this study will be used afterwards to investigate the exposure of agricultural workers (operators) using dimethoate in typical field exposure scenarios. Experimental conditions are chosen in such away that it is not expected that study participation will result in any health effect.

3 STUDY OBJECTIVE

The objective of the present study is to establish in healthy male volunteers the urinary elimination profile of dimethoate and/or its metabolites after a single oral administration of dimethoate.

4 STUDY SPONSOR

The study is sponsored by the Dimethoate Task Force located in Mannheim, Germany

5 EXPERIMENTAL DESIGN

The study is designed as a controlled, single dose, open study, in which 6 apparently healthy males, aged between 18 and 45, will participate in the study. The duration of the study will comprise 4 study days. On the first day, dimethoate will be administered to the subjects. The substance will be dissolved in a small volume of distilled water. During the study period all produced urine has to be collected and several bloodsamples will be taken at 3 study days. Prior to the study 4 visits to TNO are required, including a medical examination and a pre-selection in which fingerprick blood will be sampled on 3 days and urine has to be collected during two 24h periods

Urine will be analysed on the presence of the study substance and metabolites. Blood samples will be used for enzyme activity determination.

6 SELECTION OF SUBJECTS

Before you can participate in this study, a selection will take place. Your possible participation will depend on the results of this selection. The selection procedure will involve the following steps:

1. You will be informed about the study both orally and in writing
2. You will be asked to sign the form 'Consent to participate' (P4802 F01)
3. You will be asked to complete a health and life-style questionnaire
4. You will be examined on the basis of a list of inclusion and exclusion criteria (annex 1) This examination comprises a conversation with the physician about the completed questionnaire, assessment of height and weight, and blood and urine analysis. as an extra safety parameter blood pressure, heart rate and an electrocardiogram (ECG) will be produced. If you are eligible according to the health criteria, a further selection will take place on the basis of analysis of variation of plasma and RBC cholinesterase activity and the presence of the study substance and metabolites in collected two 24h urine samples.
5. You will be informed whether you have been selected for the study or on the reserve list.

7 CONDUCT OF THE STUDY

7.1 Time schedule and study duration

The programme of the study is summarized in Annex 2
The study will be conducted in the research centre of TNO Nutrition and Food Research at Zeist.

The study will include a 4 day period from Monday 04 August to 07 August 2003. On Monday 04 August you are expected to come to TNO **in the fasting state**; blood sampling will be performed and the study substance will be administered. Additionally, there will be an ECG recording. At around lunchtime you may leave TNO. The following day and the third day after the study substance administration, you will have to visit TNO as well (**in the fasting state**) for blood sampling, ECG recording and to deliver collected urine. At the end of the study (72 h) a blood sample (fingerprick) is taken and an additional blood (venepuncture) and an urine sample will be collected for the post-study screen. In addition, an ECG will be produced and heart rate and blood pressure will be measured. During your stay at TNO there will be a possiblity to read, to study or to watch T.V. Tea and coffee as well as bread rolls will be available for breakfast or lunch

7.2 Study substance

The study is carried out with the insecticide dimethoate. In the study a solution of a small amount dimethoate in distilled water will be prepared. The chosen dose of the study substance equals the acute

reference dose (0.03 mg/kg b.w.). This dose is considered to pose no health risk for single uptake of dimethoate via the diet e.g., due to potential residues. The acute reference dose is derived from a clinical trial in which 0.3 mg/kg b.w has been applied repeatedely for 39 days and did not cause any adverse effect. To compensate for possible interindividual differences in sensitivity a safety factor of 10 is used

7.3 Blood and urine analysis

In this study blood and urine samples will be collected. Blood will analysed for the activity of plasma- and RBC (red blood cell) cholinesterase. Therefore, these blood samples will be dispatched to and analysed by a clinical-chemistry hospital laboratory at Delft; (SSDZ) Stichting Samenwerkende Delftse Ziekenhuizen. Urine samples will be analysed for dimethoate and the dimethoate metabolites. These analysis will be conducted under the responsibility of the sponsor by a certified English laboratory (Huntingdon Life Sciences Ltd.). Therefore, urine samples will be dispatched to this laboratory. The creatinin content of urine samples will be analysed by TNO.
All blood- and urine samples will be used only for the establishment of the aforementioned analytes.

7.4 Safety and possible side effects

Owing to the low dose that will be administered in this study, not a single health effect is expected. The general safety procedures for clinical trials at TNO will be followed, however. At each visit to TNO, a well-being questionnaire has to be filled out. The medical investigator will review your answers and may contact you. During all your visits a state-registered nurse or a registered first-aider will be present in the facility.

8 POST-STUDY SCREEN

On the final study day (day 04), prior to departure from TNO you will be asked about mental and physical well-being by one of the coworkers of TNO Nutrition and Food Research and an exit interview with the medical investigator may take place. This interview may be initiated by the medical investigator, based on complaints or at your request. A physical examination will also be performed. A blood and urine sample in the fasting state will be collected for clinical chemistry; in addition an ECG will be recorded and heart rate and blood pressure will be measured.

9 ENVISAGED INCONVENIENCE

If you will participate in the study you may expect the following inconveniences:

Physical examination by medical doctor
Completion of a number of questionnaires
Eight visits to TNO (including the selection days). Usually, these visits will take 1 - 2 hours. One visit will comprise of half a day (morning)
Assessment of electrocardiogram (3x)
Collection of all urine during two periods of 24h and a period of 72h.
Drinking of a glass of water in which the study substance is dissolved.
Blood sampling by fingerprick (7-times in total on various days)
Blood sampling by venepuncture (4-times)