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**The oral bioavailability of sodium nitrite
investigated in healthy adult volunteers**

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ABBREVIATIONS

AE	Adverse Experience
Alk. Phos.	Alkaline phosphatase
ALAT	Alanine amino transferase
ANOVA	Analyses of Variance
ARO	Laboratory of Residue Analyses
ASAT	Aspartate amino transferase
AUC ₀₋₂₄	Area under the curve of a 24 hour interval
AZU	Utrecht University Hospital
b.p.m.	Beats per minute
bw	Body weight
BW	Body weight
cm	Centimetres
C _{max}	maximum concentration
dL	deciliters
ECG	Electrocardiogram
exp9	times 10 ⁹
F	female
g	gravity
gr	gram
GCP	Good Clinical Practice
gamma-GT	Gamma Glutamyl Transpeptidase
GGT	Gamma Glutamyl Transpeptidase
GLP	Good Laboratory Practice
Hb	Hemoglobin
HPIC	High Performance Ion Chromatography
hr	hour
HR	Heart rate
Ht	Haematocrit
kg	kilogram
LDH	lactate dehydrogenase
M	Male
metHb	Methaemoglobin
min	minutes
mg	milligram
mmHg	millimeters mercury pressure
Mφ	Micro-organisms
NaNO ₂	Sodium nitrite
NMN	N-methylnicotinamide
NO ₂ ⁻	Nitrite

NO ₃ ⁻	Nitrate
NOEL	No Observed Effect Level
NVIC	National Poisons Control Centre
RIVM	National Institute of Public Health and the Environment
SD	Standard Deviation
SOP	Standard Operating Procedure
t _{max}	Time to maximum concentration
t _{1/2}	Time needed to reach half of the initial plasma concentration
U/L	Units per liter
V _z	Volume of distribution
WHO	World Health Organization
WOM	Medical Ethics Committee of the Utrecht University Hospital
yrs	years
y/m/d	year/month/day

ABSTRACT

Nitrate levels in food products and drinking water tend to increase continuously in Western Countries. The toxicity of nitrate is low. However, in the proximal part of the intestinal tract, part of the nitrate is converted to nitrite, a more toxic compound. This orally formed nitrite can only perform systemic toxicity in case it is absorbed from the gastro-intestinal tract. The oral bioavailability of sodium nitrite was, therefore, determined in healthy adult volunteers. This study demonstrates that, after an overnight fast, 90 to 95 % of orally ingested sodium nitrite is absorbed from the gastro-intestinal tract. Nitrite rapidly disappeared from the plasma with an elimination half-life of approximately 30 minutes.

SUMMARY

This report describes the results of a healthy volunteers study, investigating the oral bioavailability of sodium nitrite, conducted at the National Poisons Control Centre of the Netherlands.

The study was designed as a single dose, open, randomized, three way cross-over study. Nine adult volunteers (7 females and 2 males) received 2 single, oral doses of sodium nitrite and one intravenous sodium nitrite dose, with a wash out period of 7 days between the treatments. The oral sodium nitrite doses were equal to, and half, the intravenous dose, respectively. All sodium nitrite doses were administered after an overnight fast. Adverse experiences, blood pressure, heart rate, hemoglobin concentration, the percentage of metHb in the blood and the plasma nitrite and nitrate concentration were frequently registered over a period of 24 hours following each administration of sodium nitrite.

Headache was the most frequent complaint during every treatment session. Lowering of the blood pressure accompanied by an increase of the heart rate was evident after each treatment. After intravenous administration, sodium nitrite induced a maximum percentage of metHb in the blood ranging between 8.4 to 12.2 %. Following an oral dose of sodium nitrite, equal to and half the intravenous dose, the maximum percentage of metHb ranged between 7.7 to 10.9 % and between 3.4 to 4.5 %, respectively. The maximum metHb level was reached at approximately 1.15 hrs post dose after both, intravenous administration and an equal dose of orally administered sodium nitrite. After administration of the low sodium nitrite dose the maximum concentration was reached at 0.70 hrs post dose.

The gastro-intestinal absorption of sodium nitrite was very fast. The maximum plasma nitrite concentrations were observed between 15 and 30 minutes post dose. Nitrite rapidly disappeared from the plasma. The average elimination half-life was approximately 30 minutes.

The oral bioavailability of sodium nitrite ranged between 73 and 118 % after the high sodium nitrite dose and between 70 and 112 % after the low dose of sodium nitrite.

It can be concluded that, under fasting circumstances, 90 to 95 % of orally administered sodium nitrite is absorbed from the gastro-intestinal tract.

SAMENVATTING (SUMMARY IN DUTCH)

Dit rapport beschrijft de resultaten van een vrijwilligers-onderzoek naar de orale biobeschikbaarheid van nitriet dat is uitgevoerd door het Nationaal Vergiftigingen Informatie Centrum in Nederland.

De studie had een open, 3-weg gekruiste, gerandomiseerde proefopzet. Aan negen proefpersonen (7 vrouwen, 2 mannen) werden 2 enkelvoudige, orale doseringen en een enkelvoudige, intraveneuze dosering van natriumnitriet toegediend. De orale doseringen waren, respectievelijk, 0.5 en 1 keer de intraveneuze dosering. Natriumnitriet werd steeds toegediend in nuchtere toestand, na een nachtelijke periode van vasten.

Eventuele klachten en symptomen, de bloeddruk en de hartslag, het hemoglobine gehalte, het percentage methemoglobine in het bloed en de plasma nitriet en nitraat concentraties werden frequent bepaald over een periode van 24 uur na toediening van de natriumnitriet. Hoofdpijn was de meest gerapporteerde klacht tijdens alle onderzoeks sessies. Alle natriumnitriet-behandelingen gingen gepaard met bloeddrukverlaging en een verhoging van de hartslag. Na intraveneuze toediening van natriumnitriet werden maximale methemoglobine percentages tussen 8.4 en 12.2 % gezien. Na orale natriumnitriet toediening met een dosering die gelijk was aan, en de helft van de intraveneuze doses, werden respectievelijk maximale methemoglobine percentages tussen 7.7 en 10.9 % en tussen 3.4 en 4.5 % waargenomen. De maximale metHb concentraties werden gemiddeld, respectievelijk 1.16 hr, 1.14 u en 0.70 u na toediening van de intraveneuze, hoge orale en lage orale natriumnitriet doses bereikt.

Natriumnitriet werd snel opgenomen uit het maag-darm kanaal. De maximale plasma nitriet concentratie werd gemiddeld waargenomen tussen 15 en 30 minuten na toediening van de dosis. De halfwaarde tijd van nitriet in plasma bedroeg gemiddeld circa 30 minuten.

De orale biobeschikbaarheid van nitriet varieerde tussen de 73 en 118 % na inname van de hoge natriumnitriet doses en tussen de 70 en 112 % na de lage natriumnitriet dosis.

Geconcludeerd kan worden dat, onder nuchtere omstandigheden, 90 tot 95 % van oraal toegediend natriumnitriet wordt opgenomen in het bloed.

1. INTRODUCTION

Nitrate levels in food products and drinking water tend to increase continuously in Western Countries (1). In some areas, growers, already, encounter problems because they cannot meet legal standards for the nitrate content of certain vegetables anymore. The standards for nitrate in vegetables are based on the present Acceptable Daily Intake (ADI). The present ADI, allocated to nitrate by the WHO, is 5 mg sodium nitrate/kg bw per day and is based on the no-adverse effect level in rats (2,3).

Nitrate itself is considered to be of low toxicity. Generally, the toxicity of nitrate has been ascribed to the conversion of nitrate into nitrite by bacterial reduction in the oral cavity (4,5) and proximal part of the intestinal tract (6,7).

In rats, however, nitrate conversion into nitrite is low. Healthy volunteer studies indicate that, in humans, approximately 7 % of a nitrate-dose is converted into nitrite in the oral cavity (8,9,10). The rat, therefore, seems less suitable to extrapolate the toxicity of nitrate to man.

Since the toxicity of nitrate is mainly determined by the amount of nitrate converted to nitrite, the ADI for nitrate may be derived from the conversion rate of nitrate to nitrite in humans and the no-adverse effect level for *nitrite* in rats.

The amount of nitrate converted into nitrite in the oral cavity provides information on the exposure of the gastro-intestinal tract to nitrite. This information may be useful when the reactivity of nitrite with amines in the food, which may lead to the formation of carcinogenic N-nitroso compounds, appears to be relevant for public health (i.e increased gastric cancer risk). This has not been supported by epidemiologic data yet (11,12). However, besides the gastro-intestinal exposure, the nitrite absorbed, may lead to systemic nitrite toxicity. At present no human data are available on the bioavailability of nitrite. Since the present ADI for nitrite is derived from the NOEL observed in rats which is based on systemic toxicity parameters, and since the formation of endogenous N-nitroso compounds in gastric cancer etiology has never unambiguously been shown, it is highly relevant to perform an oral nitrite bioavailability study in healthy volunteers.

In blood nitrite is rapidly oxidized by hemoglobin to yield methaemoglobin and nitrate. Calculating the oral bioavailability from the plasma-nitrite concentration is, therefore, hampered and requires a substantial nitrite dose to be administered to healthy volunteers. Therefore, the sodium nitrite doses, which were investigated for their bioavailability, in the present study, were more than 10 times the dose of nitrite which would enter the stomach via the saliva after a nitrate exposure of e.g. twice the ADI.

The acute effects of large doses nitrite, are:
methaemoglobinaemia, vasodilatation (facial redness, headache, dizziness, hypotension and palpitations) and gastro-intestinal disturbances (nausea, vomiting, abdominal cramps

and diarrhoea). At a methaemoglobin percentage of 15 % or more cyanosis may occur, between 30 and 40 % symptoms like fatigue, weakness, tachypnoea, tachycardia, headache and dizziness occur. In more severe cases of methaemoglobinaemia hypoxic convulsions, coma and other symptoms can appear, because of lack of oxygen in the tissues.

Thus, there are limitations to the nitrite dose which can safely be administered to volunteers, in a bioavailability study.

The present study was, therefore, preceded by 3 dose finding studies:

- an in vitro experiment testing the relationship between the nitrite dose and the induced methaemoglobin level in human blood samples,
- a single ascending, intravenous dose study in healthy volunteers, investigating the maximum nitrite dose which could safely be administered, i.e. inducing approximately 10% metHb and
- a single, oral, dose finding study in healthy volunteers investigating whether a substantial oral bioavailability was to be expected at the selected dose level.

The results of these experiments are reported separately (13,14,15).

Under acidic gastric conditions nitrite is unstable in the stomach (16,17). Therefore, the oral bioavailability of nitrite may depend on the nitrite dose administered. To investigate this possibility, within the limits given, two nitrite doses were selected for determination of the oral bioavailability.

The intragastric stability of nitrite not only depends on the gastric pH but also on the presence of certain compounds in the stomach (food constituents) which react with nitrite (17). In order to investigate the worst case scenario for the bioavailability, sodium nitrite was administered to the volunteers in fasting state and fasting was maintained until 4 hours post dose.

Recent experiments with nitrite in rats showed increased urinary excretion of N-methylnicotinamide, a tryptophan metabolite, possibly caused by disturbances in the amino acid metabolism of the liver. In the present study the urinary excretion of N-methylnicotinamide was also investigated. The results are reported separately (18).

This report provides a detailed description of the oral bioavailability of sodium nitrite and the nitrite-induced clinical effects and methaemoglobin concentration and also the nitrite and nitrate concentrations in plasma, in relation to the administered dose.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Study Population

Participants for the study were recruited by announcements on the bulletin boards in the buildings of the Utrecht University and through direct mailing to former participants or individuals who had notified the Department to be willing to participate in healthy volunteer studies. One month to one week before the start of the study, volunteers, willing to participate, were screened for enrolment by a pre-study medical examination. The medical examination consisted of a standard medical history questionnaire, a physical examination, 12 lead electrocardiography (using a Hewlett Packard cardiograph, type 4700 A) and non-invasive automated blood pressure measurement (using a Passport Monitor of Datascope^R). Furthermore, blood and urine samples were drawn for routine laboratory analyses.

The pre-study medical examinations were performed by the medical staff of the Department of Intensive Care and Clinical Toxicology of the University Hospital in Utrecht (AZU).

The participants were finally selected for enrolment in the study on basis of the results of the medical screening and the selection criteria as summarized in Appendix 5.

The General Practitioner was informed when his patient was participating in the study and was asked to inform the medical investigator when he disagreed on the decision to enrol the subject in the study.

If the routine blood and urine analyses revealed abnormal values, the clinical relevance was determined. If the results were considered to be of no clinical relevance the volunteer was included in the study. Otherwise, a new blood or urine sample was drawn and analysed until normal results were obtained or the volunteer was excluded from participation in the study.

After the study was completed the volunteers underwent a post-study medical examination. The volunteers were asked to report any change in their health status as compared to the pre-study medical screening. Furthermore, the physical examination and routine blood and urine investigations were repeated.

2.1.2. Products under Study

Sodium nitrite (CAS 7632-00-0) was dissolved in distilled water in an isotonic concentration of 10 mg/ml. The sodium nitrite formulation was prepared by the Pharmacy of the University Hospital in Utrecht and delivered in bottles of 100 ml each. For each

volunteer and each session a new bottle was used.

All nitrite studies (the in vitro nitrite study (13), the intravenous ascending dose study (14), the oral dose finding study (15) and the present study) were performed with sodium nitrite originating from the same batch (identification number: 99950619 42614).

During the study the bottles were stored in the dark, at room temperature.

After completion of the study one bottle of the batch was analysed by the Laboratory for Residue Analyses for the actual sodium nitrite content. The results indicated an actual sodium nitrite content of 9.7 ± 0.2 mg/ml in the bottles. The remainder of the batch will be returned to the Pharmacy.

2.2. Study Sites

The clinical part of the study was performed at the Clinical Research Unit of the National Poisons Control Centre in Utrecht, the Netherlands.

The routine laboratory analyses of blood and urine specimen obtained during pre and post-study screening were performed by the Department of Clinical Chemistry of the Utrecht University Hospital (AZU), the Netherlands.

The analyses of the plasma samples for nitrate and nitrite were performed by the Laboratory of Residue Analyses of the National Institute of Public Health and the Environment (RIVM) in the Netherlands.

The analyses of the urine samples for N-methylnicotinamide were performed by the Laboratory for Effect Assessment of the National Institute of Public Health and the Environment (RIVM) in the Netherlands.

2.3. Methods

2.3.1. Study Protocol

The study was carried out in accordance with the protocol entitled "The absolute bioavailability of an sodium nitrite dose investigated in healthy volunteers (project number 348801/04, dated 18-04-1995, revised 10-08-1995)". The study protocol was approved by the Medical Ethics Committee (WOM) of the University Hospital in Utrecht, the Netherlands.

The study was performed under the recommended principles of Good Clinical Practice for studies with medicine in the European Community (Appendix 1 and 2).

The participants in the study were fully informed, both orally and in writing, about the purpose of the study, the study design and the possible risks involved. The volunteers signed a written statement of consent (Appendix 4).

2.3.2. Study design

General

The study was designed as a single dose, open, randomized, three way cross-over study. Nine healthy adult volunteers received each of the following treatments with a washout period of 7 days between the administrations:

Treatment A = Intravenous administration of 0.12 mmol sodium nitrite per mmol hemoglobin infused over 30 minutes

Treatment B = Oral administration of 0.12 mmol sodium nitrite per mmol hemoglobin

Treatment C = Oral administration of 0.06 mmol sodium nitrite per mmol hemoglobin

In blood sodium nitrite reacts almost instantaneously with hemoglobin, to form methaemoglobin and nitrate (19). The sodium nitrite dose to be administered to the volunteers was, therefore, related to the total amount of hemoglobin, calculated to be present in the body of the individual volunteers (see below). The sodium nitrite doses to be administered during the treatments were thus expressed as an amount per mmol Hb. This way of dosing was focused on preventing an unacceptable metHb level in the blood (i.e. > 10-15 %).

The following formula was used to calculate the actual amounts of sodium nitrite to be administered to the volunteers:

$$\text{NaNO}_2 \text{ dose in mg} = A * 0.07 * \text{BW} * \text{Hb} * 69$$

in which,

A = Sodium nitrite dose in mmol per mmol hemoglobin (0.06 or 0.12).

0.07 = Factor to derive the blood volume from the body weight. On average, 70 ± 10 ml blood per kg body weight is present in the human body. Blood volume = $0.07 * \text{Body weight}$.

BW = Body weight of the volunteer, in kg, as determined at the first treatment session before administration of the sodium nitrite dose.

Hb = Hemoglobin concentration of the volunteer, in mmol/l, as determined at the first treatment session before administration of the sodium nitrite dose. The total amount of Hb in the circulation = $\text{Hb} * \text{blood volume}$.

69 = Molecular weight of sodium nitrite

The Hb concentration and the body weight determined at the first treatment session, before dosing, were used for the dose calculations during all treatment sessions.

Intravenous treatment

For intravenous administration of sodium nitrite a volumetric infusion pump, type IVAC® 560, was used. The infusion pump was connected to an intravenous cannula inserted in a forearm vein. The cannula was inserted in the forearm opposite to the arm in which the cannula for blood sampling was inserted.

Sodium nitrite was infused over 30 minutes. The infusion speed and the volume were dependent on the calculated sodium nitrite dose to be administered to the volunteer (see page 14).

Oral treatment

The sodium nitrite solution for oral administration was weighted with a Sartorius Portable balans, type PT 600, of Sartorius Instruments BV, the Netherlands.

The sodium nitrite solution was diluted with distilled water to a final weight (i.e volume) of 200 mg. The sodium nitrite doses for oral administration were equal to, and half, the intravenous dose, respectively.

Randomization

The randomization code for the treatment sequence was generated by the Pharmacy of the University Hospital in Utrecht using the software programme SYSTAT. For logistic reasons, the randomization was performed within 4 sub-blocks and 3 cases per sub-block, so that, on a particular study-date, two volunteers received an oral treatment and one volunteer an intravenous treatment with sodium nitrite.

A complete sequence randomization was not possible because the sequence of the three treatments was to be randomized over 9 volunteers (and three replacements). Since the carry over effect was accounted for by a considerable wash out period, the randomization was performed such as to balance for a possible period effect.

The sodium nitrite solution was administered to the volunteer in fasting state and fasting was maintained until approximately 4 hours post dose.

Clinical observation of the participants took place from approximately 1.5 hours before until 24 hours after administration of sodium nitrite. During this period several blood samples were taken, adverse experiences were recorded, the blood pressure and heart rate were determined and continuous electrocardiographic monitoring was performed (see Appendix 6). The volunteers stayed overnight in the Clinical Research Unit.

At each session, before administration of the sodium nitrite, a pregnancy test (TestPack Plus™ hCG-urine, ABBOTT) was performed in female volunteers and the body weight of all volunteers was determined using a Seca balance of Schinkel Medical Instruments, the Netherlands.

Diet

Three days prior to and on each investigation day the volunteers consumed a nitrite and nitrate low diet (Appendix 7). The actual food intake was registered in a diary.

On each study day the same meals were consumed during clinical observation. Between 4 and 4.5 hours post dose, lunch was served, consisting of 3 slices of bread (two with jam and one with cheese) and one glass of milk.

Between 6 and 6.5 hours post dose, tea, two slices of bread with jam and one banana were served. Between 9 and 9.5 hours post dose, a nitrate and nitrite low dinner was served consisting of white rice, haricot beans and beef ragout and a vanilla custard (vanilla vla). Breakfast was served after the 24 hour blood sample. A fixed amount of 200 ml of distilled water was administered at 1.5 and 3 hours after each sodium nitrite treatment. After the 4 hour blood sample distilled water was taken ad libitum.

Adverse Experiences

During the study, the volunteers were frequently asked about adverse experiences, in general terms. The participants were requested to report all side effects, whether related to the sodium nitrite or not.

Blood pressure and heart rate

Blood pressure and heart rate were measured with a non-invasive automated blood pressure meter (Passport Monitor[®] of Datascope) at several occasions during the day. The blood pressure and the heart rate were determined with the participant being in bed, in a slightly upright position. The volunteers remained in this position until 4 hours post dose. The time points on which the blood pressure and the heart rate were determined were specified in advance, following the scheduled procedures as presented in Appendix 6. When clinically indicated more frequent blood pressure measurements were performed.

Continuous heart rate monitoring

Participants were under continuous ECG-surveillance (lead II or III) for 4 hours following the administration of sodium nitrite (using the Passport Monitor of Datascope).

Print outs of rhythm strips were made, each session, before dosing and if clinically indicated thereafter.

Blood sampling

Blood samples of 5 ml were taken from a cannula inserted in a forearm vein. The blood sampling was performed following Standard Operating Procedures (20) with the volunteer being in supine position and without using tourniquet pressure. In general, the 24 hour blood sample was obtained by vein puncture in the fossa cubiti, using tourniquet pressure. The blood samples were collected in heparinized tubes. Immediately after collection the blood samples were introduced in the IL 282 CO-Oximeter to determine the percentage of methaemoglobin and the hemoglobin concentration. Thereafter, the samples were centrifuged for 10 minutes at 3000 g and at 4°C. The supernatant (plasma) was

transferred into polypropylene tubes within 10 minutes. The plasma samples were immediately frozen in liquid nitrogen and stored at -80°C until analyzed for the nitrate and nitrite content. The scheduled time points for blood sample collection are presented in Appendix 6.

The total amount of blood taken from each participant during the investigation (including pre- and post-study screening) was approximately 345 ml.

Urine collection

The urine collection started at 00.00 h on the investigation day and continued until 24 hours after administration of sodium nitrite. The volume of the urine portions was determined by weight with a Sartorius Portable Balans, type PT 600 of Sartorius Instruments BV, the Netherlands. The volume of the urine portion and the time of urination were registered in the Case Report Forms of the volunteers. From each urine portion two samples of 10 ml each were drawn and frozen in liquid nitrogen. Thereafter the samples were stored at -80°C until analyses for the N-methylnicotinamide content. The results of the N-methylnicotinamide analyses will be reported separately. The remainder of the urine will be stored at -80°C until the study is completed.

2.3.3. Sample Analyses

Routine laboratory analyses

During the pre and post study screening blood and urine samples were taken for routine laboratory investigations. The following parameters were determined:

In blood(plasma): Hb (cyanomethemoglobin method, NE-8000 (Sysmex)), Ht (NE-8000 (Sysmex), calculated from RBC and MCV); methaemoglobin (IL-282 CO-Oximeter (Instrumentation Laboratory)); leucocytes + differentiation (NE-8000 (Sysmex)); sodium, potassium and calcium (indirect ISE method, Hitachi 717 (Boehringer)); urea (urease-GLDH method, Hitachi 717 (Boehringer)); creatinine (alkaline picrate method, Hitachi 717 (Boehringer)); bilirubin (Jendrassik-Grof method, Hitachi 717 (Boehringer)); ASAT, ALAT, gamma-GT and LDH (IFCC method at 37°C, Hitachi 717 (Boehringer)).

In urine: qualitative urine analyses on glucose, protein, blood and leucocytes (dipstick method, seven-path test strip, Urotron RL-9 (Boehringer)).

Microscopic analyses of the urine was only performed if indicated by the results of the qualitative urine analyses (fase contrast microscopy of centrifugated urine).

The normal range of the parameters is presented in Appendix 10.

The precision of the test-results is assured by interlaboratory surveillance procedures ("ring validation" method).

(Met)hemoglobin measurements

The percentage of methaemoglobin and the hemoglobin concentration (gr/dL) were determined with an IL 282 CO-Oximeter (Instrumentation Laboratory BV, the

Netherlands) according to Standard Operating Procedures (21). The Hb concentration was measured in gr/dL and converted to mmol/l by multiplying with 0.62.

The IL 282 CO-Oximeter measures the absorbance spectra of diluted and hemolyzed blood samples at four specific wavelengths to enable differentiation between four species of hemoglobin: oxyhemoglobin, methaemoglobin, reduced hemoglobin and carboxyhaemoglobin. The total hemoglobin concentration is calculated subsequently. Within the range of measurement, the stated 95% confidence limits of accuracy are \pm 0.2 gr/dL for the Hb and \pm 1% for the metHb, respectively. The stated precision is 0.2 gr/dL and 0.5%, respectively.

The CO-Oximeter was daily calibrated on the total hemoglobin value with IL CalDye^R solution (lotno. 7412203).

Nitrate and nitrite analyses

Concentrations of nitrate and nitrite in plasma were determined at the Laboratory for Residue Analyses by means of a previously established method based on High Performance Ion Chromatography and UV detection at 208 nm (22). This method was optimized to ensure a limit of quantification for plasma nitrite which was sufficiently low. Analyses of the samples took place according to SOP ARO/414 (23). For quality assurance about 1 out of 10 samples was analysed in duplicate. Recovery experiments and analyses of blanc samples took place daily. Additional quality assurance included frequent analyses of home made quality control plasma samples with a nitrite concentration of 3.00 mg/kg and a nitrate concentration of 13.8 mg/kg.

The results of all but one of the duplicate samples were within 10% of the mean concentration of the samples. The recovery experiments showed an average recovery of 100 \pm 10% for all but one of the experiments. The lower limit of quantification was 2 mg/kg for plasma nitrate and 0.3 mg/kg for plasma nitrite.

2.3.4. Data Analyses

Toxicokinetic analyses were performed on the metHb concentration and the plasma nitrite and nitrate concentrations. For toxicokinetic analyses the computer programme Topfit 2.0 was used.

The area under the curve (AUC), terminal elimination half-life ($t_{1/2}$), maximum concentration (C_{max}), time to maximum concentration (t_{max}) and the volume of distribution (V_z) were calculated on the basis of a model independent analyses.

The AUC was calculated from the data, using the linear trapezoid rule and extrapolation to infinity from the lowest reliable data point onwards. The lowest reliable datapoint being 1.5% for metHb, 0.3 mg/kg for plasma nitrite and 2 mg/kg for plasma nitrate. The percentage of the AUC that was obtained via extrapolation, is indicated in the tables (Extrapolation AUC (%), Appendix 15, 17 and 19). For treatment comparison, the AUC

and C_{max} were adjusted to a standard sodium nitrite dose of 320 mg (C-max adjusted*, Appendix 15, 17 and 19).

Under normal circumstances, small amounts of nitrate and methaemoglobin are present in the blood. The metHb and plasma nitrate concentrations, as observed after administration of sodium nitrite, were, therefore, corrected for the baseline concentration. It was assumed that the baseline metHb and nitrate concentration in the blood reflect a steady state situation. The baseline metHb and plasma nitrate concentrations of the three study periods were, therefore, averaged and the data of every treatment period corrected for this mean baseline concentration.

The baseline plasma nitrite concentrations were in all but one cases below the detection limit of 0.1 mg/kg. The results were not corrected for a baseline concentration since there are, as yet, no evident data on nitrite being present in the blood under normal circumstances.

The volume of distribution (V_z) of nitrite, nitrate and metHb was calculated from the results obtained after intravenous administration of sodium nitrite applying the formula $V_z = \text{NaNO}_2 \text{ dose}/(\text{AUC}^*(\ln 2/t_{1/2}))$.

The terminal elimination half-life of metHb was calculated from a time point of twice t_{max} onwards, being from 2.5 hrs post dose onwards for treatment A and B and from 1.75 hrs post dose, onwards for treatment C. MetHb values below 1.5% were not included in the calculation of the terminal elimination half-life.

The half-life of plasma nitrite was calculated from a timepoint of twice t_{max} onwards, being 1.17 hrs post dose for treatment A and a variable timepoint for treatments B and C, depending on the observed t_{max} .

The half-life of plasma nitrate was calculated from a timepoint of 5 hrs post dose onwards for each of the treatments. In a number of cases, two half lifes could be obtained from the plasma nitrate data as indicated in Appendix 19.

For statistical analyses the computer programme SAS/STAT 6.08 for Windows 3.10 was used. The significance of a difference in the kinetic parameters between the reference treatment A and the oral treatments B and C was calculated with the two tailed student t-test for paired data. The Hb concentration was tested for a period and treatment effect by ANOVA procedure.

Data are presented as mean \pm SD unless stated otherwise, a p-value below 0.05 was considered to be significant.

2.4 Archiving

All study documents, including this report, will be kept on file in the GCP-archives of the Department of Medical Toxicology of the National Poisons Control Centre of the Netherlands for a period of at least 15 years.

3. RESULTS

3.1 General

No major protocol violations occurred. In cases that the study procedures were not in full compliance with the protocol this was registered. The deviations from the protocol are summarized in Appendix 3. The deviations to the protocol had no impact on the outcome of the study.

3.2 Pre-Study Medical Screening

Fourteen volunteers underwent a pre-study medical examination between September 11 and September 19, 1995. The demographic characteristics of these volunteers are summarized in Appendix 8. Three volunteers were not eligible: one male volunteer (0 BZ) because the laboratory test results showed an elevated bilirubin level of $33 \mu\text{mol/l}$ (normal range $< 17 \mu\text{mol/l}$), one female volunteer (0 LH) because the dipstick urine analyses was repeatedly positive for blood and one female volunteer (0 AS) because of a medical history of bronchitis. Two female volunteers (10 PV and 11 HH) entered the study as possible replacements. They did not receive treatment because all participants completed the study.

Seven female and two male, caucasian, volunteers entered and completed the study. The mean age of the volunteers, who participated in the study was 22.9 ± 1.5 years. The mean weight of the participating volunteers was 67.8 ± 3.3 kg (range 62-72 kg) and their height was 173.3 ± 5.8 cm (range 166-182 cm).

All volunteers who entered the study were non-smokers and did not take any concurrent medication except for oral contraceptives.

The medical history and the physical examination of the participating volunteers did not reveal abnormalities which were considered to have implications for their participation in the study. Volunteer 8 RV suffered from a common cold. The physical examination revealed enlarged submandibular lymph nodes and the haematology test results showed elevated leucocytes and a shift in the differential leucocyte count from lymphocytes towards granulocytes. Since the common cold subsided within a week and the repeated haematology test showed normal results the volunteer was included in the study.

The routine laboratory test results of the blood showed minor abnormalities. Except for the elevated leucocyte count observed in volunteer 8 RV and the elevated bilirubin level for volunteer 0 BZ, these were all judged by the physician as of no clinical relevance.

In general, dipstick urine analyses yielding negative or weakly positive test results were considered to be of no clinical importance. The dipstick urine analyses of volunteer 9 EL was positive for leucocytes and strongly positive for blood. Microscopic examination of

the urine showed much amorf salts. Since the volunteer was in the middle of her menstruation period the findings were considered to be due to the menstruation. The results of the vital signs and routine laboratory tests are tabulated in Appendix 10.

3.3 Post-Study Medical Screening

The post-study medical examinations were performed between October 18 and November 1, 1995. The results are presented in Appendix 10. No changes in health were observed. The routine laboratory test results showed minor abnormalities. Except for the test result of the urine of volunteer 9 EL these were all judged by the physician as of no clinical relevance. The slightly elevated leucocyte count of volunteer 6 MR matched a minor, ongoing common cold.

The dipstick urine analyses of volunteer 9 EL was positive for leucocytes and strongly positive for blood. Microscopic examination of the urine showed 5 to 10 leucocytes and 0 to 4 erythrocytes per field. Blood in the urine was also observed at the pre-study screening. However, during the pre-study screening the volunteer was in the middle of her menstruation period, but during the post-study medical examination she was not. Repeated efforts were made to contact the volunteer for a second urine test, without success. The clinical relevance of the test result could, therefore, not be established. However, in most females these findings turn out to be clinically irrelevant. The General Practitioner of the volunteer was, nevertheless, informed.

The blood in the urine was considered not to be study-related, since it was already observed during the pre-study medical examination and it was considered not have influenced the outcome of the study.

3.4 Study Dates and Treatment Sequence

The clinical part of the study was performed between September 25 and October 20, 1995. The actual dates on which sodium nitrite was administered and the treatment sequence are presented in Appendix 9.

3.5 Sodium Nitrite Doses

The actual amount of sodium nitrite, administered to each volunteer during the treatment sessions, is presented in Table 3.5.1. The amount, to be administered, was dependent on the body weight and the blood-hemoglobin concentration (Hb) of the volunteers (see section 2.3.2.). The body weight and Hb are, therefore, presented here as well.

The sodium nitrite doses administered during treatments A and B ranged from 290 to 380 mg and for treatment C from 140 to 190 mg.

TABLE 3.5.1

Volunteer number	Weight (kg)	Hb (mmol/l)	Treatment A NaNO ₂ (mg)	Treatment B NaNO ₂ (mg)	Treatment C NaNO ₂ (mg)
1 TN	71.0	8.40	350	350	170
2 MP	70.0	9.38	380	380	190
3 IS	62.0	8.02	290	290	140
4 DL	68.0	8.27	330	330	160
5 JB	65.0	8.27	310	310	160
6 MR	68.0	7.65	300	300	150
7 SG	64.5	8.02	300	300	150
8 RV	69.5	8.95	360	360	180
9 EL	62.0	8.21	290	290	150

3.6 Adverse Experiences

During the preceding pilot experiments with sodium nitrite, side-effects such as headache and nausea were observed. It was therefore communicated in advance to the volunteers in the present study that these side effects could occur during the study.

The adverse experiences (AEs) as reported by the individual volunteers, during the treatment sessions, are summarized in Appendix 11. In general, AEs which are known to be related to sodium nitrite were classified as possibly related. AEs reported by the volunteer in more than one treatment sessions were classified as definitely (yes) related to the testproduct. AEs present before administration of the testproduct were classified as not related. With respect to the treatment-related AEs, no distinction was made between nitrite and its metabolite, nitrate.

No serious AEs were observed during any of the treatment sessions. Most AEs were of a mild intensity except for one headache of moderate intensity and two cases of moderate nausea/vomiting. All of the reported AEs were known to be related to sodium nitrite.

Headache was the most frequent complaint during each of the treatments.

The time interval between administration of sodium nitrite and the appearance of complaints varied considerably, ranging from 15 minutes until 15 hours post dose. The cases of nausea, however, all occurred within 30 minutes after administration of sodium nitrite.

Volunteers 7 SG and 9 EL were the only volunteers who did not report adverse experiences related to sodium nitrite during any of the treatment sessions.

A summary of the AEs is given in Table 3.6.1.

TABLE 3.6.1

Treatment	Adverse Experience	Frequency of Adverse Experience	Number of Volunteers
Treatment A	Headache / Head discomfort Dizziness Nausea Taste of salt All Adverse Experiences	9 1 1 1 12	5 1 1 1 7
Treatment B	Headache Malaise Weakness Nausea Vomiting All adverse Experiences	4 1 1 2 1 9	4 1 1 2 1 6
Treatment C	Headache All adverse Experiences	6 6	4 4

According to the number of AEs the intravenous administration of sodium nitrite was least well tolerated (7 volunteers reported 12 AEs) followed by high dose, oral administration of sodium nitrite (6 volunteers reported 9 AEs) and low dose, oral administration of sodium nitrite (4 volunteers reported 6 AEs).

The following adverse experiences are reported in more detail:

Volunteer 4 DL experienced a mild headache after low dose, oral treatment with sodium nitrite. The headache started 10.45 hours post-dose and became of moderate intensity at 14.45 hours post-dose. Because of sleeplessness, due to the headache, paracetamol (acetaminophen), 500 mg, was administered during the night-shift. Thereafter, the headache subsided. The paracetamol was considered not to have influenced the study results.

Volunteer 5 JB became nauseous within 20 minutes after high dose, oral administration of sodium nitrite. The nausea subsided after 5 minutes as she vomited. The vomit was collected and consisted of a blanc, viscose, solution weighting 9 gram. Unfortunately, the vomit was not analysed for the sodium nitrite content. It cannot be excluded that a small amount of the ingested sodium nitrite dose was present in the vomit. Statistical tests on relevant kinetic parameters of the metHb concentration in the blood and the nitrite and nitrate concentration in the plasma were therefore performed both, with and without including the data of volunteer 5.

During intravenous administration of sodium nitrite volunteer 5 JB reported postural head

discomfort and postural dizziness. These complaints first became apparent as she came out of bed after having been in a supine position for a period of 4 hours following the sodium nitrite administration. The complaints recurred each time in standing position and lasted several hours. The postural dizziness was probably due to mild orthostatic hypotension as a result of vasodilatation. At the time of the complaints the plasma nitrite concentration was already below the detection limit of 0.1 mg/kg. It is however, unlikely that nitrate is responsible for the effect, since intravenous sodium nitrate doses as high as 9.5 gram do not lead to side effects (24).

Volunteer 6 MR became nauseous after both, intravenous administration of sodium nitrite and high dose, oral administration of sodium nitrite. During intravenous administration the nausea occurred within 29 minutes after the start of the infusion, was of moderate intensity and lasted only 3 minutes. After oral administration, the nausea occurred within 25 minutes, was of mild intensity and lasted 30 minutes. Since the nausea occurred both, after intravenous administration as well as after oral administration of sodium nitrite it is likely that the nausea is a systemic effect of nitrite instead of a local gastric effect.

Volunteer 7 SG caught a cold just before the first treatment session (low dose, oral administration of sodium nitrite). Since the common cold was of a mild intensity and was not accompanied by fever, the scheduled treatment sessions were not postponed. The complaints subsided after the second treatment session.

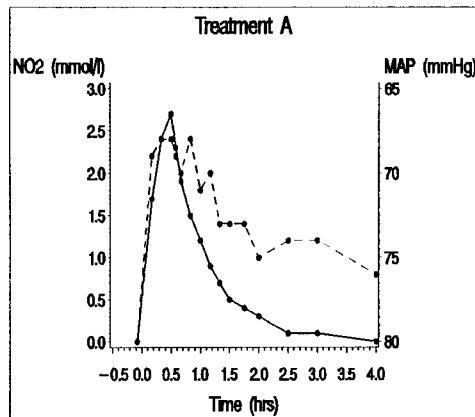
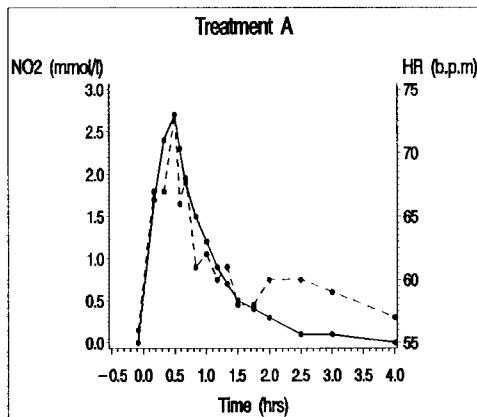
Volunteer 8 RV experienced a taste of salt in his mouth, 40 minutes after the start of the intravenous administration of sodium nitrite. This is probably due to the active transport of the nitrate-salt (and possibly nitrite) from the blood to the oral cavity.

3.7. Vital Signs

Nitrites are known to lower the blood pressure through vasodilatation (25, 26, 27). To prevent complaints of orthostatic hypotension we decided to keep the volunteers, in our study, in a recumbent position for 4 hours following the administration of the sodium nitrite dose. The interference of physical activity on the blood pressure and heart rate is minimal in supine position. The blood pressure and heart rate as observed during the first 4 hours post dose, therefore, most optimally reflect the effect of nitrite on the blood pressure. The blood pressure and the heart rate data of the individual volunteers are tabulated in Appendix 12a. Appendix 12b graphically presents the Mean Arterial Pressure (MAP) and the heart rate of the individual volunteers. The Mean Arterial Pressure is defined as the mean of the systolic (S) and twice the diastolic (D) blood pressure ($MAP = (2*D + S)/3$). Despite the great variability in the data, which can often be observed for blood pressure and heart rate measurements, all volunteers showed a clear increase of the heart rate within the first hour after each of the treatment sessions (except for volunteer 6 MR during treatment C). For most volunteers also, lowering of the MAP was evident within the first hour after treatment.

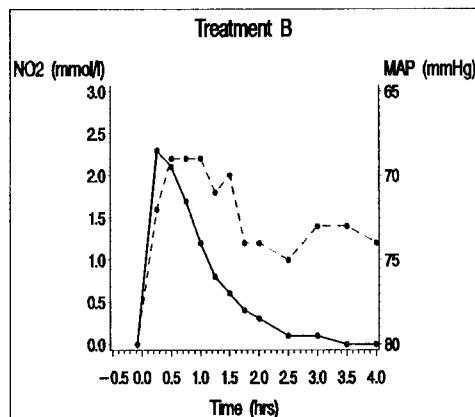
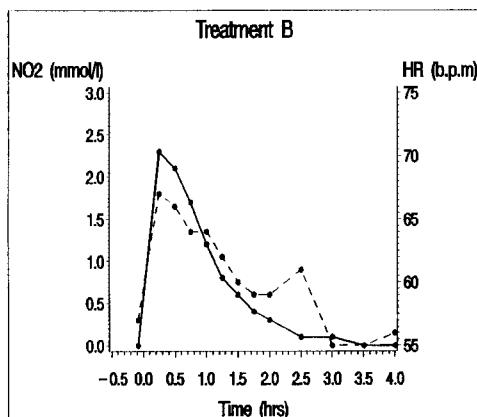
Figures 3.7.1, 3.7.2 and 3.7.3 present the averaged heart rate and MAP of the nine

volunteers, as observed over the first 4 hours post-dose, in relation to the plasma nitrite concentration. To enhance interpretation, the values on the axis of the MAP are presented in descending order.

**FIGURE 3.7.1**

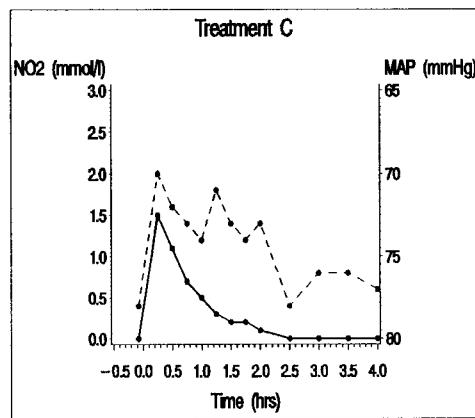
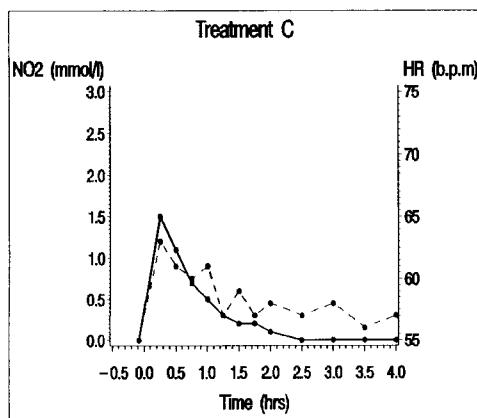
Left:
 (-----) = Heart rate
 (—) = Plasma Nitrite

Right:
 (-----) = MAP
 (—) = Plasma Nitrite

**FIGURE 3.7.2**

Left:
 (-----) = Heart rate
 (—) = Plasma Nitrite

Right:
 (-----) = MAP
 (—) = Plasma Nitrite

**FIGURE 3.7.3**

Left:
 (-----) = Heart rate
 (—) = Plasma Nitrite

Right:
 (-----) = MAP
 (—) = Plasma Nitrite

The average data clearly show lowering of the MAP accompanied by compensatory heart rate acceleration within 15 minutes after the start of the intravenous infusion or oral administration of sodium nitrite. The maximum effect is observed between 15 and 30 minutes post dose. The averaged heart rate increased from 56 to 73 b.p.m. after treatment A and from 57 to 67 b.p.m. and from 55 to 63 b.p.m. after treatments B and C, respectively. Simultaneously, the averaged MAP decreased from 80 to 68 mmHg after treatment A and from 80 to 69 mmHg and from 78 to 70 mmHg after treatments B and C, respectively. The changes in the heart rate and MAP subsided, more or less, in 2 to 3 hours. The averaged data suggest that the MAP did not fully return to the baseline value within 4 hours after treatments A and B. However, in the data of the individual volunteers this trend is not so apparent.

A tendency towards a dose-effect relationship can be observed. The intravenous treatment (treatment A) and the high dose oral treatment (treatment B) being most effective to induce the above mentioned changes in the heart rate and MAP and the low dose, oral administration (treatment C) being least effective. For the individual volunteers such a dose-effect relationship was not always so prominent (see the figures in Appendix 12b and 12c).

The graphic presentation of the heart rate in relation to the plasma nitrite concentration shows that the heart rate increases and decreases simultaneously with the plasma nitrite concentration, i.e. without notable lag-time. On average, the heart rate changed approximately 4 to 5 b.p.m. with every mg/kg change in the plasma nitrite concentration.

The MAP also decreases simultaneously with the increase of the plasma nitrite concentration. However, there seems to be a lag time before the MAP returns to the baseline level.

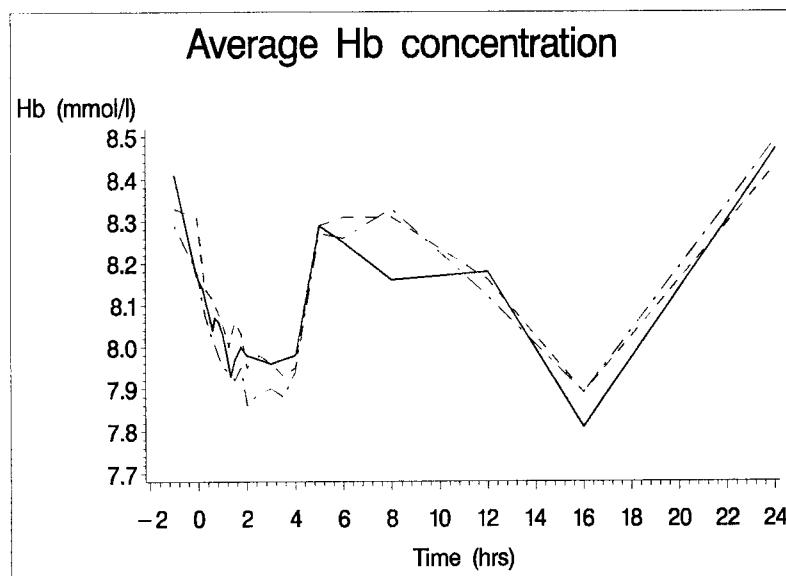
3.8 Hemoglobin Concentrations

The hemoglobin concentration (Hb) of the individual volunteers, as observed on several occasions following each of the treatments is tabulated and graphically presented in Appendix 13a and 13b, respectively. The average Hb concentration of the nine volunteers as observed over 24 hours, following each of the treatments, is presented in figure 3.8.1.

Despite standard procedures for the blood sampling, considerable variation in the Hb concentration was observed; the difference between the lowest and the highest measured Hb concentration within one person, on one day, ranged from 0.44 to 0.93 mmol/l (measurements performed under tourniquet pressure excluded).

The graphics from the individual volunteers, as well as the average figure, consistently show that, during the day, the measured Hb concentrations are lowest between 1 and 4 hours after administration of the sodium nitrite and also during the night (16 hour blood sample).

We regard the observed variability as posture related. The volunteers continuously remained in supine position from one hour before until 4 hours following each sodium nitrite treatment and also during the night. It is well known that the hydrostatic pressure acting on the circulatory system of the legs, in upright position, can cause fluid filtration to the tissues and reduce the blood volume by as much as 15 to 20 percent (28).

**FIGURE 3.8.1**

(—) = Treatment A
 (---) = Treatment B
 (- · -) = Treatment C

This fluid filtration can raise the haematocrit and, therefore, the hemoglobin concentration of the blood to a considerable extent. In supine position the fluid is reabsorbed into the circulation and thus lowering the hemoglobin concentration again.

The individual volunteers also show a considerable day-to-day variability in the Hb concentration; the difference between the lowest and the highest mean Hb concentration ranging from 0.13 to 0.55 mmol/l (= comparison of the average Hb concentration observed between 1 and 4 hours post dose). Both, female and male volunteers, showed day-to-day variability in the Hb. The menstruation was, therefore, not (solely) responsible for the effect.

A two-way ANOVA was performed on the average Hb concentration observed between 1 and 4 hours post dose as dependent variable and treatment and period as independent. Treatment ($p=0.956$) as well as period ($p=0.661$) had no significant influence on the observed Hb concentrations. The issue was not further addressed.

Because of the controlled conditions during the first 4 hours post dose (supine position, fasting, controlled fluid intake) and the fairly constant Hb concentration during this period, the average Hb concentration between 1 and 4 hours post dose was assumed to be the best representative for converting the percentage of metHb to a methaemoglobin concentration in mmol/l.

3.9 Methaemoglobin Data

The percentage of methaemoglobin in the blood of the individual volunteers, as observed on several occasions following each of the treatments, is tabulated in Appendix 14a and graphically presented in Appendix 14b. The methaemoglobin concentration (mmol/l) was calculated from the percentage of methaemoglobin using the average Hb concentration as observed between 1

and 4 hours post dose for the conversion. The calculated methaemoglobin concentration is tabulated in Appendix 14a.

The percentage of metHb was measured twice before each treatment session. The baseline metHb ranged from 0.1 to 0.8 %. During intravenous administration of sodium nitrite the maximum metHb concentration of 0.802 ± 0.090 mmol/l (mean \pm SD, range in percentages: 8.4 to 12.2%) was reached at 1.16 ± 0.13 hrs (range 1.00 to 1.33 hrs) after the start of the intravenous infusion. Thereafter, the methaemoglobin concentration decreased approximately exponentially with time, with an elimination half-life of 1.21 ± 0.15 hrs.

After the oral treatments B and C, the maximum methaemoglobin concentration in the blood was 0.727 ± 0.093 mmol/l (range in percentages: 7.7 to 10.9 %) and 0.309 ± 0.028 mmol/l (range in percentages: 3.4 to 4.5 %), respectively. The maximum metHb concentration was observed at 1.14 ± 0.25 hrs and 0.70 ± 0.16 hrs post dose, respectively. The half-life for the metHb-reduction averaged to 1.13 ± 0.13 for treatment B and to 1.07 ± 0.15 hrs for treatment C.

The kinetic parameters for metHb are summarized in Appendix 15. Note that all kinetic parameters were determined from the concentration-time data which were corrected for the mean baseline-metHb concentration, observed over 3 treatment periods. The Student-*t*-test (paired) showed significant differences for the $AUC_{0-\infty}$ and C_{max} of both oral treatments in comparison to the intravenous treatment ($p < 0.01$). The t_{max} for metHb occurred significantly earlier after the low oral dose of sodium nitrite than after both, the intravenous dose and the high oral dose ($p = 0.0001$).

3.10 Plasma Nitrite Concentrations

The plasma nitrite concentrations of the individual volunteers, as observed on several occasions following each of the treatments are tabulated and graphically presented in Appendix 16a and 16b, respectively. The kinetic parameters derived from the plasma nitrite concentrations are summarized in Appendix 17.

The baseline plasma nitrite concentrations were in all, but one, cases below 0.1 mg/kg. For volunteer 3 IS the baseline plasma nitrite concentration was twice 0.1 mg/kg. The intravenous sodium nitrite dose was administered over 30 minutes. The peak-plasma nitrite concentration was reached between 30 and 35 minutes after the start of the infusion and amounted to 2.9 ± 0.6 mg/kg. Nitrite rapidly disappeared from the plasma. Between 2.5 and 4 hours post dose the nitrite concentrations became below 0.1 mg/kg for all volunteers. The half-life of plasma-nitrite, as determined after intravenous administration of sodium nitrite, was 0.53 ± 0.10 hrs.

After oral administration, nitrite was rapidly absorbed from the gastro-intestinal tract.

For five out of 9 volunteers the peak plasma nitrite concentration was observed at 15 minutes post dose, after both oral treatments. For the other volunteers the peak concentration was observed at 30 or 45 minutes post dose.

After the high oral dose of sodium nitrite (treatment B) the average peak plasma nitrite

concentration was 2.6 ± 0.7 mg/kg. After the low oral dose of sodium nitrite (treatment C) the average peak plasma nitrite concentration was 1.6 ± 0.6 mg/kg.

The half-life of plasma nitrite, as calculated from the oral data, was 0.44 ± 0.10 hrs after treatment B and 0.42 ± 0.14 hrs after treatment C. The half-life as observed after low dose oral administration of sodium nitrite differed significantly from the intravenous treatment ($p=0.047$). The significance of this difference may be doubtful, because of the low number of datapoints on which the calculation of the half-life is based. As yet there is no evidence which suggests that the elimination of nitrite may follow non-linear kinetics.

The oral bioavailability of sodium nitrite (F) is calculated applying the formula: $F = \text{NaNO}_2\text{-dose}_{\text{iv}} / \text{AUC}_{\text{nitrite-iv}} * \text{AUC}_{\text{nitrite-po}} / \text{NaNO}_2\text{-dose}_{\text{po}}$. The average bioavailability (F) of the highest oral sodium nitrite dose, calculated this way, is 0.97 ± 0.18 (range 0.73 to 1.18, volunteer 5 excluded). The average bioavailability of the lower oral sodium nitrite dose is 0.89 ± 0.14 (range 0.70 to 1.12). The difference in bioavailability between the high and low, oral sodium nitrite dose was not significant ($p=0.21$).

The volume of distribution (V_z) of nitrite was calculated from the intravenous data, applying the formula: $V_z = \text{NaNO}_2\text{-dose} / (\text{AUC}_{\text{nitrite}} * (\ln 2 / t_{1/2}))$ and. The V_z thus calculated was 93 ± 19 liters.

In Appendix 16 it is indicated with a dash if the plasma samples, in which the plasma nitrite concentration was measured, were haemolytic. Also the degree of haemolyses (= colour-intensity) is indicated. The apparent nitrite concentration is clearly increased in moderate and strongly haemolytic blood samples (e.g. volunteer 1, treatment A, 0.30 hr; volunteer 2, treatment B, 8.13 hr; volunteer 7, treatment B, 16.00 hr; volunteer 8, treatment C, 16.08 hr).

3.11 Plasma Nitrate Concentrations

The plasma nitrate concentrations of the individual volunteers, as observed on several occasions following each of the treatments are tabulated and graphically presented in Appendix 18a and 18b, respectively. The kinetic parameters derived from the plasma nitrate concentrations are summarized in Appendix 19.

Nitrate is normally present in the blood. For toxicokinetic analyses, therefore, the plasma nitrate concentrations, as observed after administration of sodium nitrite, were corrected for the baseline nitrate concentration of the plasma. In the present study, however, the baseline nitrate concentrations were mostly below the limit of quantification of 2 mg/kg (range < 2 to 4.4 mg/kg). Although concentrations below the limit of quantification are less reliable, it seemed more appropriate to correct for these less reliable nitrate concentrations than correcting for the determination limit of 2 mg/kg. Correcting for the limit of quantification would imply a definite "overcorrection" of the data. The fasting plasma nitrate concentration was measured twice before each treatment session and the baseline nitrate concentrations as observed over three study periods were averaged.

The maximum concentration of nitrate, was reached at 1.75 ± 0.40 hours after the start of the intravenous infusion of sodium nitrite and 1.53 ± 0.34 hours and 1.64 ± 0.60 hours after oral ingestion of the high and low dose of sodium nitrite, respectively.

In a number of cases the plasma nitrate profile showed two or three peaks around its maximum concentration (see Appendix 18b). This is probably due to analytical or experimental variability. The maximum plasma nitrate concentration was 12.4 ± 1.3 mg/kg after intravenous administration and 12.1 ± 1.8 mg/kg and 5.9 ± 1.1 mg/kg after oral administration of the high and low dose of sodium nitrite, respectively.

The half-life of plasma nitrate was calculated from the plasma nitrate concentrations as observed from 5 hours post dose onwards. After intravenous administration of sodium nitrite the half-life of plasma nitrate was 8.2 ± 2.3 hours. After the high and low, oral, dose of sodium nitrite the half-life of plasma nitrate was 7.2 ± 2.1 hours and 8.7 ± 2.5 hours (volunteer 1 excluded), respectively. None of the kinetic parameters differed significantly between the intravenous treatment and the oral treatments.

For most volunteers the plasma nitrate levels were still slightly elevated at 24 hours post dose.

4. DISCUSSION

4.1 Study Design

The objective of the present study was to investigate the absolute, oral bioavailability of nitrite. From preceding pilot experiments with volunteers it was clear that high nitrite doses would be necessary to obtain plasma nitrite concentrations which were sufficiently above the limit of quantification of the analytical method for the quantification of plasma nitrite (0.3 mg/kg). However, the rapid intravenous administration of these high doses caused a pronounced decrease of the blood pressure. This was assumed to be related to the C_{max} (14). In the present study, therefore, the infusion time was prolonged from 10 to 30 minutes. Because, the C_{max} after oral administration was attained between 15 and 30 minutes post dose, the concentration-time profiles of plasma nitrite, after intravenous and oral treatment, were comparable and adequate for bioavailability calculations.

To obtain good concentration-time data of a compound, which is rapidly eliminated from the blood, such as nitrite, the study must be carefully designed. Important in this study was therefore the intensive blood sampling scheme. In order to obtain the maximum plasma nitrite concentration, the blood samples were taken with a 5 to 10 minutes interval around the end of the intravenous infusion of sodium nitrite. Because it was uncertain when the peak plasma nitrite concentration would occur after oral administration of sodium nitrite, the blood samples were taken with an interval of 15 minutes for a two hour period after the intake of sodium nitrite. The maximum plasma nitrite concentration was attained at 15 or 30 minutes after oral administration, thus, in some volunteers a blood sample was obtained just before and in others directly after the actual peak concentration. The part of the AUC of nitrite, after oral administration, that was missed for reasons the blood sampling scheme did not cover the actual peak plasma nitrite concentration, was probably small because for both oral treatments a high bioavailability was obtained.

In the present study the reaction of nitrite with hemoglobin continued until the blood samples were centrifugated and the plasma separated from the blood cells. With the currently available method and optimized procedure a minimum time of 15 minutes was needed for the handling of the blood samples before the plasma was eventually frozen in liquid nitrogen and consequently, possible reactions prevented. Gaining time could only have been achieved with more advanced technics. To slow down the decay of nitrite, the blood samples were kept at 4°C during the centrifugation and separation of the plasma from the blood cells. It is obvious that the ongoing elimination of nitrite resulted in decreased nitrite concentrations in the samples. This may have contributed to the high apparent volume of distribution of nitrite, of about 93 liters, which was calculated from the formula. When the $AUC_{nitrite}$ is underestimated, due to the decreased plasma nitrite concentrations, the V_z will be overestimated.

Because nitrite is a highly reactive compound its apparent volume is easily overestimated. The structural characteristics of nitrite and nitrate are very close. Therefore, it is plausible to assume that the real volume of distribution of both ions is similar. The volume of distribution of nitrate

was determined in a previous healthy volunteer study after an oral sodium nitrate dose and was circa 33 liters (10). Wagner et al. calculated a volume of distribution of nitrate of 21 liter in a volunteer study after oral sodium nitrate administration (29).

The time-interval between blood sampling and subsequent freezing of the plasma in liquid nitrogen varied from 15 to 20 minutes. The length of the time interval for the handling of the blood samples was regarded as randomly distributed over the samples. It may, however, have influenced the outcome on the oral bioavailability of nitrite. Since the individual bioavailability factors, all, were within a relatively narrow range from each other, without major outliers, it is likely that the decay of nitrite during the blood sample handling had minor effect on the outcome of the bioavailability of nitrite.

Overall can be concluded that, the study design proved to be adequate.

4.2 Oral Bioavailability

To our knowledge this is the first study to describe the bioavailability of nitrite in human volunteers. For risk analyses, doses within the physiological range, are preferred for bioavailability testing. However, as explained above, this was not totally possible for technical reasons. The administered doses in the present study were about 10 times the dose of nitrite which would enter the stomach via the saliva, after a nitrate dose of two times the ADI.

Two oral sodium nitrite doses, of 0.06 mmol/mmol Hb and 0.12 mmol/mmol Hb, respectively, were tested for their bioavailability. Both oral doses were highly bioavailable and did not differ significantly with respect to their bioavailability ($p=0.38$). From the present study the conclusion can be drawn, that under fasting conditions, nitrite is highly bioavailable (90-95%) from an aqueous solution.

Most of human nitrite exposure originates from the conversion of salivary nitrate. Each time, after swallowing, small amounts of saliva with nitrite will enter the stomach during the day. In vitro studies have shown that nitrite is unstable in an acidic gastric environment (16,17). The bioavailability of nitrite may, therefore, depend on the extent to which nitrite is oxidized within the stomach at low pH. In view of this, it is important to mention that the sodium nitrite doses, that were tested in the present study, were administered in an aqueous solution with a final volume of 200 ml. Such a volume may lead to a quick passage of the sodium nitrite through the stomach. In the present study a substantial decay of nitrite, due to its passage through the acidic stomach, was not observed. Nevertheless, the oral bioavailability of the small amounts of nitrite, entering the stomach under physiological circumstances, when the gastric pH is low, may be overestimated if the bioavailability from the present study is used in risk assessments. However, in cases of hypochlorhydria such as pernicious anaemia, atrophic gastritis or chronic antacids treatment high gastric pH and consequently high gastric nitrite concentrations have been observed (30,31). Furthermore, we showed that sodium nitrate administration to volunteers with an artificially increased gastric pH, due to omeprazole (proton pump inhibitor) treatment, is associated with increased nitrite concentrations in the gastric juice, as compared to the condition of a normal gastric pH (10). Therefore, in cases of hypochlorhydria the bioavailability of nitrite may approach the results as found in the present study.

It should be notified that the bioavailability of nitrite was tested under fasting conditions, in the present study. After a meal, nitrite may react with food constituents in the stomach. Thus, it is possible that in the presence of food the bioavailability of nitrite is decreased. It is, however, important to realise that most of human nitrite exposure originates from the conversion of salivary nitrate, that is excreted from the blood into the saliva after intake of nitrate rich foods. Because of the delay the nitrite, formed in the saliva, may enter the stomach when the stomach is empty again. For risk analyses, therefore, we assume the bioavailability of nitrite, as investigated in this study, under fasting conditions, is relevant.

The half-life of nitrite in plasma, as determined after intravenous administration of sodium nitrite was approximately 30 minutes (range 25 to 43 minutes). This is the first report to describe the rate of elimination of plasma nitrite, in humans *in vivo*. Ignarro reported the half life of nitrite, *in vitro*, in the presence of oxyhemoglobin at 37 °C and 22°C, to be approximately 45 and 75 minutes, respectively (32). The observed difference between the *in vivo* and *in vitro* rate of elimination of nitrite may be due to the experimental conditions of the *in vitro* study and/or due to interindividual variability in the rate of formation of metHb within the erythrocytes. Another possibility is that a second route of elimination contributes to the lower elimination half-life of nitrite *in vivo*, i.e. salivary and/or urinary excretion of nitrite or a second metabolic pathway, for example, reactivity of nitrite with myoglobin.

Haemolytic blood samples contained higher nitrite levels in the plasma. It is not likely that the nitrite in the plasma originated from the haemolysed erythrocytes because the percentage of metHb in these blood samples was low. The higher nitrite levels may be due to white blood cell contamination of the plasma. Leucocytes can generate nitrite through the arginine-nitric oxide-NO₂ pathway (33). Radisavljevic et al. determined the intracellular nitrite concentration of the human monocyte/macrophage cell line U-937, cultured in RPMI 1640 culture medium containing fetal bovine serum, bicarbonate, penicillin/streptomycin, and fungizone, and found a nitrite level of 0.66 ± 0.08 μmol/L/10⁶ Mφ (34). The higher nitrite levels in our plasma samples may be explained by a substantial white blood cells contamination of the plasma. When the 'buffy coat' is not properly separated from the plasma in the centrifuged blood samples this may increase the nitrite content of the plasma considerably when the cells disrupt during immediate freezing of the plasma samples in liquid nitrogen. For future kinetic studies with nitrite, it may be important to pay attention to preventing white blood cell contamination and, consequently, nitrite contamination of plasma samples.

4.3 Formation of Methaemoglobin

In the present study, the methaemoglobin concentration was measured as an alternative variable to calculate the oral bioavailability of sodium nitrite from ($F = \frac{Dose_{nitrite\ iv}}{Dose_{nitrite\ po}} \times \frac{AUC_{metHb\ iv}}{AUC_{metHb\ po}}$). For the oral sodium nitrite dose that was equal to the intravenous dose, the bioavailability of nitrite, calculated this way, ranged from 71 to 99 %. Thus, supporting the observed high bioavailability of nitrite calculated from the plasma nitrite data. It also indicates that the route of exposure to nitrite, intravenous versus oral, has no influence on the induced percentage of metHb.

For sodium nitrite doses, not equal to the intravenous dose, the bioavailability of nitrite may be determined from the methaemoglobin concentrations if the kinetics of the reaction between nitrite and hemoglobin, and the reduction of methaemoglobin, are first order and, therefore, are proportional to the administered sodium nitrite dose, and will be hampered if this is not the case. A volunteer study in which three ascending, intravenous, sodium nitrite doses were tested, suggests that the kinetics of the reaction between nitrite and hemoglobin and/or the reduction of methaemoglobin are probably not first order and thus depend on the administered dose (14). Doubling of the sodium nitrite dose led to an $AUC_{nitrite}$ which was twice as high. However, the AUC_{metHb} increased more than twice after doubling the sodium nitrite dose. Consequently, the bioavailability of the lower oral sodium nitrite dose, in the present study, may be underestimated when the bioavailability is calculated from the metHb concentrations (56%, Appendix 15).

The metHb formation as well as the nitrite kinetics and the results on the bioavailability of nitrite will be incorporated in a Physiologically Based Pharmacokinetic model (PBPK-model) which was previously developed to describe the fate of nitrate in the human body (35).

5. CONCLUSIONS

There is widespread concern over exposure to nitrate from dietary sources and its potential risk to human health. The toxicity of nitrate is low. However, in the proximal part of the gastro-intestinal tract, nitrate is partly converted to nitrite. Nitrite is considered to be more toxic.

If a risk assessment is performed after oral nitrate exposure, it is important to take into account the conversion of nitrate into nitrite and, consequently, the oral bioavailability and toxicity of nitrite. In a previous volunteer study (10) we found that 7% of nitrate was converted into nitrite.

From the present study it can be concluded that, under fasting conditions, sodium nitrite, administered in an aqueous solution, in doses up to 380 mg, is almost entirely bioavailable. The elimination half-life of nitrite in plasma was approximately 30 minutes. The two oral sodium nitrite doses that were administered in the present study, of 0.06 mmol/mmol Hb and 0.12 mmol/mmol Hb, which is approximately 160 mg per person for the lower dose and 320 mg per person for the higher dose, induced a methaemoglobinaemia of approximately 4 and 9%, respectively. This was accompanied by a dose dependent increase in adverse effects such as headache, lowering of the blood pressure and increased heart rate.

For the risk analyses of nitrate a Physiologically Based Pharmacokinetic model (PBPK-model) was developed (35). The results of the present study will be used for calibrating and validating the developed PBPK-model, with respect to the nitrite kinetics and the formation of methaemoglobin. The interaction between the PBPK-model and the experimental work will direct the design of future studies with nitrate and/or nitrite to be performed within the framework of the risk analyses of nitrate exposure. In the end the PBPK-model will be tuned in the way that it is an adequate tool to perform risk analyses for different scenarios of possible nitrate exposure.

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APPENDIXES

Appendix 1. Statement of GCP Compliance

The undersigned hereby certify that the clinical phase of the study, as described herein, was performed in accordance with the recommended principles of Good Clinical Practice for studies with medicine in the European Community (EC doc. III/3976/88 FINAL).

This report provides a complete, correct and faithful record of the results obtained. The study was performed by the clinical investigator J. Kortboyer, MD.

Name: J.M. Kortboyer, MD (clinical investigator)

Laboratory: Unit National Poisons Control Centre, RIVM

Date: 13-06-1997

Signature:



Name: J. Meulenbelt, MD PhD (principal investigator)

Laboratory: Unit National Poisons Control Centre, RIVM

Date:

Signature:



Appendix 2. Quality Assurance Statement

The undersigned hereby certifies that the clinical phase of the study, as described herein, was performed in accordance with the recommended principles of Good Clinical Practice for studies with medicine in the European Community (EC doc. III/3976/88 FINAL).

This report provides a complete, correct and faithful record of the results obtained.

QA inspections were performed on the following dates:

1995: April 18th
September 27th and 29th
1996: April 26th
May 1st, 2nd, 3rd

QA inspection reports to the study director and management are dated:

1995: April 18th
1996: May 3rd

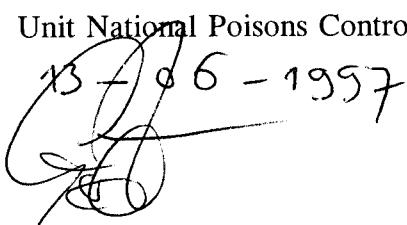
QA audits of this report were performed on the following dates:

1997: April 14 - 24th
May 2nd, 12th
June 11th

QA audits of this report were reported to the study director and management on:

1997: May 13th
June 11th

Substitute Quality Assurance Manager

Name: Drs. G.A. van Zoelen
Laboratory: Unit National Poisons Control Centre, RIVM
Date: 13-06-1997
Signature: 

Appendix 3 Deviations from the Protocol

Due to an administrative error, the Department of Clinical Chemistry omitted to determine the lactatedehydrogenase level (LDH) in the blood samples that were obtained at the pre-study medical screening. Alkaline Phosphatase was measured instead. The volunteers were enroled in the study, nevertheless. At the first treatment session, just before administration of the sodium nitrite, an additional blood sample was drawn for LDH measurement.

Volunteer 1 TN and 2 MP weighted 71 and 72 kg, respectively. They were included in the study although they did not meet the selection criterion of a body weight between 60 and 70 kg. The slightly out of range body weights of volunteers 1 TN and 2 MP were considered to be acceptable.

Due to inattention the blood pressure and heart rate of volunteer 4 DL were not measured at 30 minutes after treatment C.

In a number of cases the blood pressure measurement was repeated for confirmation. Only the first measurement is indicated in the tables unless the result was considered unreliable. In that case the second measurement was included in the table.

Due to blood sampling difficulties, the first pre-dose blood sample during treatment B was omitted for volunteer 9 EL. The missing data did not influence the results of the study.

A number of blood samples were obtained somewhat later then the scheduled time. The actual times on which the blood samples were drawn are mentioned in the relevant Appendices.

The 24 hrs post dose blood samples of volunteers 4, 5 and 6, during the first treatment session, were left in the centrifuge (at 4 °C) for 3 hours before the plasma was transferred to another tube and frozen in liquid nitrogen. Since nitrate is stable in blood and the nitrite level is expected to be below the lower limit of quantification, at this timepoint, the results of the nitrate/nitrite analyses were considered not to have been influenced.

A blood sample of volunteer 8 RV, obtained at 1 hour post dose during treatment A, was thawed by accident after initial freezing in liquid nitrogen. Since laboratory tests showed that nitrite is stable in plasma, this was considered of no influence on the results (personal communication Laboratory for Residue Analyses).

An additional blood sample was obtained from volunteer 4 DL at 0.45 hr post dose during treatment B because the first sample was spilled on the floor.

The blood samples for nitrate and nitrite analyses of volunteers 1 TN and 2 MP, obtained at 12 hours post dose during treatments A and B, respectively, got lost.

The 12 hour blood samples of volunteers 1, 2 and 3 obtained during treatment C, A and B, respectively, showed plasma nitrate concentrations not in line with expectations. Since, these blood samples where all taken on the same date, a systematic error is likely to have occurred. These data were, therefore, omitted when calculating the AUC.

The nitrate and nitrite low diet was not followed accurately by some volunteers. In all but one cases this did not influence the baseline nitrite and nitrate concentration of the blood, i.e. the plasma nitrate concentration remained below the determination limit of 2 mg/kg. Volunteer 8 RV consumed potato-products the evening before treatment A and B. The mean baseline plasma nitrate concentration was 2.5 and 2.4 mg/kg during treatments A and B, respectively, versus 2.1 mg/kg during treatment C. Considering the small differences in the baseline plasma nitrate concentration, the influence of the diet was accepted as negligible.

In a number of cases the diet, during the investigation days, was adjusted on the first treatment day, i.e. less/more bread. These changes were then maintained during the subsequent treatment sessions.

Volunteer 1 TN applied 3 nose drops of epinephrine at 23:45 hr, the day before treatment B was given, because of a nose congestion. This was considered not to have influenced the study results.

On September 27, 1995 the Sartorius Portable Balans type PT 600, used to determine the weight of the sodium nitrite doses and the volume of the urine samples, became defect. On September 29 the weight of the sodium nitrite dose (volunteer 7 SG) was determined with a calibrated Mettler balans from the Pharmacy of the Utrecht University Hospital. From October 4, 1995 onwards the weight of the sodium nitrite doses was determined with a calibrated Sartorius Portable Balans type 1204 MP.

The Sartorius Portable Balans type PT 600 was not recently calibrated before the start of the study. To determine its calibration status, the weight of 14 bottles, determined in advance with the Sartorius Portable Balans PT 600, was compared to the weight, as determined afterwards, with the Sartorius Portable Balans type 1204 MP. The observed differences in weight amounted to 0.22 (low weight) to 0.93% (high weight) of the weights as determined with the Sartorius Portable Balans type 1204 MP. The introduced error in the administered sodium nitrite doses was considered to be negligible. The results were not corrected for the observed difference.

Appendix 4 Written Informed Consent Form

Appendix II

INFORMED CONSENT FORMULIER

**Afd. IC1 en Klinische Toxicologie van het Academisch Ziekenhuis Utrecht
Nationale Vergiftigingen Informatie Centrum van het RIVM**

Ondergetekende verklaart een exemplaar te hebben ontvangen van de "Informatie voor Deelnemers" betreffende het onderzoek getiteld:

"Onderzoek naar de opname van natrium nitriet in het bloed vanuit het maag-darm kanaal: een bio-beschikbaarheidsstudie (datum 18-04-1995/ revisiedatum 10-08-1995)".

Ondergetekende heeft een mondelinge toelichting op dit informatieformulier gekregen en is in de gelegenheid geweest iedere gewenste vraag te stellen.

Ondergetekende verklaart dat zij/hij, na kennis te hebben genomen van bovengenoemde informatie, als vrijwilliger wil deelnemen aan bovengenoemd onderzoek.

Ondergetekende verklaart dat hij/zij naar eer en geweten alle vragen gesteld tijdens de medische keuring zal beantwoorden en geen informatie bewust heeft achtergehouden.

Afgesproken is dat zij/hij zich gedurende de totale onderzoeksperiode zal houden aan de opzet van het onderzoek en aan de restricties zoals beschreven in de schriftelijke informatie voor vrijwilligers.

Voorts verklaart de vrijwilliger dat zij/hij in een periode van 30 dagen voorafgaand aan en tijdens dit onderzoek niet deelneemt aan enig ander vrijwilligsonderzoek.

Aan ondergetekende is medegedeeld dat bij vragen en problemen gedurende 24 uur per dag, 7 dagen per week direct kontakt kan worden opgenomen met de onderzoekers via het Academisch Ziekenhuis Utrecht, afdeling Intensice Care-I en Klinische Toxicologie, tel. 030-507330 (tijdens kantooruren) en het Nationaal Vergiftigingen Informatiecentrum, tel. 030-748888 (buiten kantooruren).

Voorts is ondergetekende medegedeeld dat zij/hij zonder opgave van reden op elk moment deelname aan het onderzoek kan staken.

Ondergetekende gaat accoord met inzagerecht van persoonlijke (medische) gegevens door medewerkers van de afdeling IC-1 en Klinische Toxicologie en medewerkers van inspecterende instanties

Naam :

Voornamen :

Geborendatum :

Adres :

Postcode + Woonplaats :

Telefoonnummer :

Utrecht,(datum en jaar)

Handtekening vrijwilliger: Naam en handtekening onderzoeker:

.....

Appendix 5 Selection Criteria

Selection criteria as applied for enrolment of volunteers in the oral bioavailability study of sodium nitrite

Inclusion criteria

- female/male
- age 18-35 years
- weight between 60 and 70 kg and within the normal range of the Metropolitan Height and Mass Tables (Appendix V)
- willing to give Written Informed Consent
- healthy as judged by a physician from the medical history, physical examination, electrocardiography and routine laboratory blood and urine analyses

Exclusion criteria

- smoking
 - chronic drug treatment (especially antacids) or any drug treatment within one week before the start of the study. Except for oral contraceptives.
 - drug abuse
 - more than 3 units of alcohol per day
 - blood donation within 30 days before the start of the study
 - participation in another drug study within 30 days before the experiment or during the study
 - pregnant or lactating females
 - mental illness
 - any chronic illness
 - family history positive for pernicious anaemia
 - family history positive for G6PD-deficiency or original inhabitants of the mediterannee
 - excessive pyrosis or history of gastritis or gastric or duodenal ulcer
 - urine incontinence or frequent cystitis
 - abnormal dietary habits as judged by a physician
 - anaemia
-

Appendix 6 Flow Chart of Scheduled Study Procedures

Scheduled Procedures during Intravenous administration of sodium nitrite

Clock-Time	Scheme-Time	Scheduled Procedure
07.30 h	-1.50	Pregnancy test, Body Weight, Inserting an intravenous cannula
08.00 h	-1.00	Blood sampling
08.00-08.55 h	-1.00 - -0.08	Connecting Chest-electrodes for continuous heart rate monitoring, Inserting another intravenous cannula, Blood Pressure, Adverse Experiences, Completing blanc urine collection
08.55 h	-0.08	Blood sampling
09.00-09.30 h	0.00 - 0.50	Administration of sodium nitrite
09.10 h	0.17	Blood sampling, Blood Pressure, Adverse Experiences
09.20 h	0.33	Blood sampling, Blood Pressure, Adverse Experiences Removing intravenous cannula
09.30 h	0.50	Blood sampling, Blood Pressure, Adverse Experiences
09.35 h	0.58	Blood sampling, Blood Pressure, Adverse Experiences
09.40 h	0.67	Blood sampling, Blood Pressure, Adverse Experiences
09.50 h	0.83	Blood sampling, Blood Pressure, Adverse Experiences
10.00 h	1.00	Blood sampling, Blood Pressure, Adverse Experiences
10.10 h	1.17	Blood sampling, Blood Pressure, Adverse Experiences
10.20 h	1.33	Blood sampling, Blood Pressure, Adverse Experiences
10.30 h	1.50	Blood sampling, Blood Pressure, Adverse Experiences, Drinking 200 ml distilled water
10.45 h	1.75	Blood sampling, Blood Pressure, Adverse Experiences
11.00 h	2.00	Blood sampling, Blood Pressure, Adverse Experiences
11.30 h	2.50	Blood sampling, Blood Pressure, Adverse Experiences
12.00 h	3.00	Blood sampling, Blood Pressure, Drinking 200 ml distilled water Adverse Experiences
13.00 h	4.00	Blood sampling, Blood Pressure, Adverse Experiences Removing chest-electrodes
13.00-13.30 h	4.00-4.50	Lunch
14.00 h	5.00	Blood sampling, Blood Pressure, Adverse Experiences
15.00 h	6.00	Blood sampling, Blood Pressure, Adverse Experiences
15.00-15.30 h	6.00-6.50	Tea
17.00 h	8.00	Blood sampling, Blood Pressure, Adverse Experiences
18.00-18.30 h	9.00-9.50	Dinner
21.00 h	12.00	Blood sampling, Blood Pressure, Adverse Experiences
22.00 h	13.00	Tea
01.00 h	16.00	Blood sampling, Blood Pressure, Adverse Experiences
09.00 h	24.00	Blood sampling, Blood Pressure, Adverse Experiences Removing intravenous cannula Completing urine collection Breakfast, Departure from Research Unit

Appendix 6 Flow Chart of Scheduled Study Procedures
 (continued)

Scheduled Procedures during oral administration of sodium nitrite

<u>Clock-Time</u>	<u>Scheme-Time</u>	<u>Scheduled Procedure</u>
07.30 h	-1.50	Pregnancy test, Body Weight, Inserting an intravenous cannula
08.00 h	-1.00	Blood sampling
08.00-08.55 h	-1.00 - -0.08	Connecting Chest-electrodes for continuous heart rate monitoring, Blood Pressure, Adverse Experiences, Completing blanc urine collection
08.55 h	-0.08	Blood sampling
09.00 h	0.00	Administration of sodium nitrite
09.15 h	0.25	Blood sampling, Blood Pressure, Adverse Experiences
09.30 h	0.50	Blood sampling, Blood Pressure, Adverse Experiences
09.45 h	0.75	Blood sampling, Blood Pressure, Adverse Experiences
10.00 h	1.00	Blood sampling, Blood Pressure, Adverse Experiences
10.15 h	1.25	Blood sampling, Blood Pressure, Adverse Experiences
10.30 h	1.50	Blood sampling, Blood Pressure, Adverse Experiences Drinking 200 ml distilled water
10.45 h	1.75	Blood sampling, Blood Pressure, Adverse Experiences
11.00 h	2.00	Blood sampling, Blood Pressure, Adverse Experiences
11.30 h	2.50	Blood sampling, Blood Pressure, Adverse Experiences
12.00 h	3.00	Blood sampling, Blood Pressure, Adverse Experiences, Drinking 200 ml distilled water
12.30 h	3.50	Blood sampling, Blood Pressure, Adverse Experiences
13.00 h	4.00	Blood sampling, Blood Pressure, Adverse Experiences Removing chest-electrodes
13.00-13.30 h	4.00-4.50	Lunch
14.00 h	5.00	Blood sampling, Blood Pressure, Adverse Experiences
15.00 h	6.00	Blood sampling, Blood Pressure, Adverse Experiences
15.00-15.30 h	6.00-6.50	Tea
17.00 h	8.00	Blood sampling, Blood Pressure, Adverse Experiences
18.00-18.30 h	9.00-9.50	Dinner
21.00 h	12.00	Blood sampling, Blood Pressure, Adverse Experiences
22.00 h	13.00	Tea
01.00 h	16.00	Blood sampling, Blood Pressure, Adverse Experiences
09.00 h	24.00	Blood sampling, Blood Pressure, Adverse Experiences Removing intravenous cannula Completing urine collection Breakfast, Departure from Research Unit

Appendix 7 Nitrate and nitrite low diet (English version)

The following diet instructions were handed out to the volunteers, participating in the nitrite bioavailability study.

VEGETABLES

A large part of daily nitrate is provided by vegetables. The nitrate-content of the vegetables can vary enormously. The diet instructions given here are based on mean nitrate concentrations in various vegetables. Because the nitrate content of the vegetables can vary enormously it is always important to eat different vegetables every day.

A Vegetables containing more than 1000 mg nitrate per kg product. These vegetables are **forbidden**

endive	black radish
celery	red beetroots
chinese cabbage	lettuce
chervil	beetroot
turnip cabbage	spinach
paksoi	cabbage
purslain	fennel
turnip tops	watercress
radish	

B Vegetables containing between 500 and 1000 mg nitrate per kg product. **Limited use** of these vegetables is permitted. The day before administration of sodium nitrite these vegetables should not be eaten.

cauliflower
curly kale
paprika
leek
sliced beans

C Vegetables containing less than 500 mg nitrate per kg product. These vegetables can be eaten **without restriction**.

artichokes	tauge
asparagus	tomato
aubergine	savoy cabbage
broccoli	butter beans
courgette	sprouts
green peas	broad beans
peas	onions
cucumber	carrots
turnip rooted celery	white cabbage
maize	belgian endive
mushrooms	sauer kraut
padded peas	
rhubarb	
red cabbage	

POTATOES

Limited use of potatoes is permitted. This also accounts for other potato-products such as chips and french fries. The day before administration of sodium nitrite these products should not be eaten.

RICE AND PASTE

These products can be eaten **without restriction**.

BREAD

Bread and bread-products can be eaten **without restriction**.

MEAT AND FISH

Fish and fresh meat can be eaten **without restriction**.

MEAT-PRODUCTS/MEAT-DELICACIES

In these products nitrate and nitrite are used as preservatives. Therefore, only limited use of these products is permitted.

The day before administration of sodium nitrite the following products should not be eaten.

bacon

ham

smoked beef

smoked sausage

MILK, EGGS AND DAIRY-PRODUCTS

These products can be eaten **without restriction**.

FRUIT

Fruit can be eaten **without restriction**.

DRINKS

The use of potable water from a private well is **forbidden**.

Consumption of vegetable juices is **forbidden**.

All other drinks can be consumed **without restriction**.

For the duration of the experiment alcohol consumption, with a maximum of three glasses a day, is permitted.

Adapted from: "Nitraatinname van de Nederlandse bevolking op basis van de Voedselconsumptiepeiling". van Loon A.J.M. and van Klaveren J.D., RIVM-report 90.18, april 1990, National Institute of Public Health and the Environment, The Netherlands.
"Nitrates and nitrites in foodstuffs: health protection of consumers", Schuddeboom L.J., Council of Europe Press, 1993.

Appendix 8 Table of Demographic Data

Demographic data obtained during the pre-study medical screening

Volunteer nr.	Gender	Age (yrs)	Height (cm)	Weight (kg)	Comment
0 BZ *	M	24	186	66	Excluded
0 AS *	F	23	177	67	Excluded
0 LH *	F	21	166	65	Excluded
1 TN	F	24	173	71	
2 MP	M	24	174	72	
3 IS	F	20	179	62	
4 DL	F	24	169	69	
5 JB	F	25	174	67	
6 MR	F	22	178	69	
7 SG	F	22	165	66	
8 RV	M	22	182	70	
9 EL	F	23	166	64	
10 PV *	F	27	175	62	Replacement
11 HH *	F	24	163	61	Replacement
Mean		22.9	173.3	67.8	
SD		1.5	5.8	3.3	
Min		20	166	62	
Max		25	182	72	

* = Excluded from descriptive statistics

Appendix 9 Table of Study Dates and Treatment Sequence

Study dates and treatment sequence of the volunteers participating in the oral bioavailability study of sodium nitrite.

Treatment A = single dose, intravenous administration of 0.12 mmol sodium nitrite per mmol Hb infused over 30 minutes

Treatment B = single dose, oral administration of 0.12 mmol sodium nitrite per mmol Hb

Treatment C = single dose, oral administration of 0.06 mmol sodium nitrite per mmol Hb

Appendix 10 Test results of the Pre- and Post-study Medical Screening

Table of Vital Signs

Volunteer number	Pre / Post Study	Date (d/m/y)	Blood Pressure Supine systolic/diastolic (mmHg) heart rate (b.p.m.)	Blood Pressure Standing systolic/diastolic (mmHg) heart rate (b.p.m.)	ECG (normal/abnormal)		
1 TN	Pre Post	18/09/95 19/10/95	118/72 119/66	60 68	121/83 124/75	95 85	normal normal
2 MP	Pre Post	12/09/95 18/10/95	121/75 113/68	72 78	124/79 113/71	81 102	normal normal
3 IS	Pre Post	11/09/95 18/10/95	102/55 118/43	47 51	123/64 118/49	56 63	normal normal
4 DL	Pre Post	11/09/95 18/10/95	111/66 119/61	71 71	122/70 120/69	72 93	normal normal
5 JB	Pre Post	13/09/95 19/10/95	107/55 96/47	47 48	109/65 118/66	65 62	normal normal
6 MR	Pre Post	11/09/95 26/10/95	126/78 121/67	75 65	119/80 119/72	98 92	normal normal
7 SG	Pre Post	13/09/95 19/10/95	118/61 114/69	52 77	112/67 132/66	62 101	normal normal
8 RV	Pre Post	18/09/95 19/10/95	122/69 140/72	66 77	130/72 127/71	82 84	normal normal
9 EL	Pre Post	15/09/95 01/11/95	118/63 109/60	51 49	115/87 99/64	64 81	normal normal
10 PV	Pre	18/09/95	109/62	76	109/73	98	normal
11 HH	Pre	26/09/95	94/44	47	101/55	57	normal
0 BZ	Pre	19/09/95	127/67	62	129/75	74	normal
0 AS	Pre	18/09/95	125/70	60	125/82	65	normal
0 LH	Pre	13/09/95	132/68	88	132/87	101	normal

**Appendix 10 Test results of the Pre- and Post-study Medical Screening
(continued)**

Table of Hematology test results

Volunteer number	Pre / Post Study	Date (d/m/y)	Normal Range:	Hemoglobin (M: 8.6–10.7 F: 7.4–9.6 Units: (mmol/l))	Hematocrite (M: 0.41–0.55 F: 0.36–0.46 (l/l))	Leucocytes (Exp9/L)	Eosinophiles < 5 %	Basophiles < 2 %	Granulocytes 40–72 %	Lymphocytes 20–45 %	Monocytes 3–10 %
1 TN	Pre Post	18/09/95 19/10/95	9.0 7.9	0.43 0.38	5.4 5.6	1 1	1 1	1 1	53 65	42 30	3 3
2 MP	Pre Post	12/09/95 18/10/95	10.0 9.2	0.44 0.41	6.2 5.2	8 * 7 *	1 1	1 1	56 59	30 28	5 5
3 IS	Pre Post	11/09/95 18/10/95	8.3 8.4	0.40 0.40	5.7 6.3	1 1	1 1	1 1	70 72	25 23	3 3
4 DL	Pre Post	11/09/95 18/10/95	8.1 8.1	0.37 0.38	5.7 5.5	2 2	1 0	1 0	59 55	36 40	2 * 3
5 JB	Pre Post	13/09/95 19/10/95	8.1 7.7	0.39 0.36	4.1 4.5	2 1	1 1	1 1	61 64	32 32	4 2 *
6 MR	Pre Post	11/09/95 26/10/95	8.0 7.9	0.38 0.38	9.6 10.9 *	4 1	0 0	0 0	62 70	30 27	4 2 *
7 SG	Pre Post	13/09/95 19/10/95	8.1 8.0	0.39 0.38	6.2 6.6	1 1	1 0	1 0	62 62	33 33	3 4
8 RV	Pre (repeat) Post	18/09/95 25/09/95 19/10/95	9.6 9.0 9.4	0.46 0.43 0.43	10.4 * 8.7 5.8	1 1 2	0 0 1	0 0 1	81 * 71 62	15 * 23 29	3 5 6
9 EL	Pre Post	15/09/95 01/11/95	8.0 7.6	0.40 0.37	6.5 6.3	1 3	0 1	0 1	66 61	30 31	3 4
10 PV	Pre	18/09/95	8.5	0.39	4	2	1	1	60	34	3
11 HH	Pre	26/09/95	7.7	0.37	8.5	2	1	1	63	32	2 *
0 BZ	Pre	19/09/95	10.2	0.47	6.6	11 *	1	1	59	25	4
0 AS	Pre	18/09/95	8.0	0.39	8.2	4	0	0	71	21	4
0 LH	Pre	13/09/95	8.3	0.40	9.7	2	1	1	62	33	2 *

* = out of normal range

**Appendix 10 Test results of the Pre- and Post-study Medical Screening
(continued)**

Table of Clinical Chemistry test results

Volunteer number	Pre / Post Study	Date (d/m/y)	Normal Range: Units: (mmol/l)	Urea (mmol/l)	Creatinine (mmol/l)	Sodium (mmol/l)	Potassium (mmol/l)	Calcium (mmol/l)	Bilirubin (micromol/l)	AF (U/L)	GGT (U/L)	ASAT (U/L)	ALAT (U/L)	LDH (U/L)
1 TN	Pre	18/09/95	3.3	114	141	4.4	2.42	13	52	14 *	23	24	-	-
	Pre	25/09/95	4.8	92	139	4.0	2.25	8	-	14 *	19	17	465	417
	Post	19/10/95	4.5	91	145	4.4	2.43	10	65	24	21	23	-	-
2 MP	Pre	25/09/95	5.1	100	143	3.9	2.32	16	-	21	18	21	416	454
	Post	18/10/95	3.7	90	143	3.8	2.31	7	37 *	34	19	23	-	-
3 IS	Pre	11/09/95	25/09/95	3.8	98	141	3.7 *	2.29	10	-	30	17	19	462
	Post	18/10/95	2.9 *	58	142	3.8	2.25	5	52	16	19	15	-	-
4 DL	Pre	11/09/95	27/09/95	2.7 *	64	143	4.0	2.30	7	-	15	18	16	397
	Post	18/10/95	3.3	80	139	3.9	2.31	8	41	23	20	30	425	-
5 JB	Pre	13/09/95	27/09/95	3.1	81	140	4.0	2.21	8	-	26	25	32	553
	Post	19/10/95	3.0	80	146	3.6 *	2.34	8	66	30	17	18	-	-
6 MR	Pre	11/09/95	27/09/95	3.6	82	142	3.7 *	2.32	7	-	21	15	11	333
	Post	26/10/95	2.8 *	70	139	3.9	2.24	6	49	18	16	19	-	-
7 SG	Pre	13/09/95	29/09/95	3.1	76	142	3.9	2.27	7	-	20	17	24	406
	Post	19/10/95	3.0	76	140	4.0	2.34	17 *	78	22	17	20	-	-
8 RV	Pre	18/09/95	Pre	3.9	80	142	4.1	2.30	17 *	-	19	17	24	350
	Post	25/09/95	4.2	79	142	4.0	2.48	15	42	21	18	20	-	-
9 EL	Pre	15/09/95	Post	5.1	81	142	3.9	2.48	16	-	18	16	13	381
	Pre	06/10/95	Post	3.9	85	139	4.3	2.32	11	63	24	27	-	-
10 PV	Pre	18/09/95	Pre	4.6	72	143	3.6 *	2.33	11	-	38	23	31	389
	Post	26/09/95	3.7	78	143	3.8	2.41	33 *	49	16	17	19	-	-
11 HH	Pre	19/09/95	0 BZ	2.9	68	142	3.8	2.27	8	46	18	19	25	-
	Pre	18/09/95	0 AS	5.3	67	140	3.8	2.27	11	57	13 *	17	12	-
0 LH	Pre	13/09/95	*	-	-	-	-	-	-	-	-	-	-	-

* = out of normal range

**Appendix 10 Test results of the Pre- and Post-study Medical Screening
(continued)**

Table of Urine Analyses test results

Volunteer number	Pre / Post Study	Date (d/m/y)	Acidity (pH) Range: (4.5–7.8)	Dipstick Urine Analyses			Microscopic Analyses of the Urine		
				Normal	Albumin	Glucose	Leucocytes	Blood	Nitrite
1 TN	Pre Post	18/09/95 19/10/95	7	w.pos neg	neg	w.pos neg	neg	neg	-
2 MP	Pre Post	12/09/95 18/10/95	5 6.5	neg w.pos	neg neg	w.pos neg	neg	neg	-
3 IS	Pre Post	11/09/95 18/10/95	- 5	neg neg	neg neg	neg	neg	neg	-
4 DL	Pre Post	11/09/95 18/10/95	6 6.5	w.pos neg	neg	w.pos w.pos	neg	w.pos w.pos	-
5 JB	Pre Post	13/09/95 19/10/95	6.5 5	neg neg	neg neg	w.pos w.pos	neg	neg	-
6 MR	Pre Post	11/09/95 28/10/95	6.5 6	neg w.pos	neg neg	w.pos w.pos	neg neg	neg pos	-
7 SG	Pre Post	13/09/95 19/10/95	7 6	neg w.pos	neg neg	w.pos w.pos	neg neg	neg w.pos	-
8 RV	Pre Post Post (repeat)	18/09/95 19/10/95 02/11/95	7 6 7	neg w.pos neg	neg neg neg	w.pos w.pos neg	neg neg neg	w.pos w.pos neg	-
9 EL	Pre Post	15/09/95 01/11/95	5 5	w.pos w.pos	neg neg	pos pos	st. pos st. pos	st. pos st. pos	0–4
10 PV	Pre	18/09/95	5	w.pos	neg	neg	w.pos	-	5–10
11 HH	Pre	26/09/95	8	neg	neg	neg	neg	-	0–4
0 BZ	Pre	19/09/95	6	neg	neg	neg	neg	-	-
0 AS	Pre	18/09/95	7	neg	neg	neg	neg	-	-
0 LH	Pre (repeat)	13/09/95 19/09/95	7 7	w.pos neg	neg neg	w.pos w.pos	st. pos st. pos	st. pos st. pos	0–4 0–4

neg = negative; pos = positive; w.pos = weakly positive; st.pos = strongly positive

Appendix 11 Table of Adverse Experiences

Adverse Experiences as reported after single dose, intravenous, administration of 0.12 mmol NaNo₂ per kg body weight to nine healthy adult volunteers (Treatment A).

Adverse Experience	Volunteer number	Time since start infusion (hrs.min)	Duration (hrs.min)	Intensity	Related to testproduct
Headache	1 TN	1.35	8.00	mild	yes
	2 MP	2.45	11.15	mild	yes
	3 IS	0.30	0.43	mild	possible
	3 IS	11.55	2.00	mild	possible
	4 DL	4.50	1.00	mild	yes
	5 JB	0.30	0.41	mild	yes
	5 JB	6.00	4.20	mild	yes
Head discomfort	3 IS	2.02	4.53	mild	possible
Head discomfort, postural	5 JB	4.00	2.00	mild	possible
Dizziness, postural	5 JB	4.00	3.20	mild	possible
Nausea	6 MR	0.29	0.03	moderate	yes
Taste of salt	8 RV	0.40	0.20	mild	possible

Appendix 11 Table of Adverse Experiences
 (continued)

Adverse Experiences as reported after single dose, oral, administration of 0.12 mmol NaNo₂ per kg body weight to nine healthy adult volunteers (Treatment B).

Adverse Experience	Volunteer number	Time started after treatment (hrs.min)	Duration (hrs.min)	Intensity	Related to testproduct
Headache	1 TN	0.25	4.55	mild	yes
	2 MP	3.50	2.10	mild	yes
	4 DL	3.05	2.40	mild	yes
	6 MR	4.25	2.50	mild	possible
Malaise	1 TN	0.40	1.00	mild	possible
Weakness	5 JB	9.50	5.30	mild	possible
Nausea	5 JB	0.20	0.05	moderate	yes
	6 MR	0.25	0.30	mild	yes
Vomiting	5 JB	0.25	0.01	moderate	yes
Common cold	7 SG	predose	continuing	mild	no

Appendix 11 Table of Adverse Experiences
 (continued)

Adverse Experiences as reported after single dose, oral, administration of 0.06 mmol NaNo₂ per kg body weight to nine healthy adult volunteers (Treatment C).

Adverse Experience	Volunteer number	Time started after treatment (hrs.min)	Duration (hrs.min)	Intensity	Related to testproduct
Headache	1 TN	0.20	23.05	mild	yes
	4 DL	10.45	2.15	mild	possible
	4 DL	14.45	1.40	moderate	possible
	5 JB	0.15	0.25	mild	yes
	5 JB	5.30	0.25	mild	yes
	6 MR	9.10	1.00	mild	possible
Common cold	7 SG	predose	continuing	mild	no

Appendix 12a Table of Vital SignsTreatment A = Intravenous administration of 0.12 mmol NaNO₂ /mmol Hb

Volunteer number		1 TN			2 MP			3 IS		
Scheduled Time (hr)	*Scheme Time (hr)	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg) (b.p.m.)	Heart rate	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg) (b.p.m.)	Heart rate	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg) (b.p.m.)	Heart rate
8.55	-0.08	-0.68	116 / 71	54	-0.75	109 / 64	57	-0.07	100 / 57	46
9.10	0.17	0.23	114 / 67	72	0.20	94 / 50	67	0.20	99 / 45	54
9.20	0.33	0.43	108 / 68	72	0.37	92 / 53	65	0.38	104 / 45	55
9.30	0.50	0.58	113 / 62	66	0.53	96 / 50	76	0.55	102 / 44	59
9.35	0.58	0.67	112 / 60	62	0.62	88 / 63	65	0.67	111 / 38	55
9.40	0.67	0.78	114 / 59	64	0.70	91 / 58	71	0.75	103 / 56	52
9.50	0.83	0.92	115 / 64	63	0.85	95 / 60	62	0.88	101 / 42	52
10.00	1.00	1.10	115 / 68	65	1.03	106 / 47	68	1.03	103 / 45	52
10.10	1.17	1.23	118 / 69	65	1.20	99 / 52	59	1.23	104 / 44	56
10.20	1.33	1.48	113 / 68	64	1.37	97 / 62	60	1.37	101 / 56	47
10.30	1.50	1.63	113 / 70	62	1.53	96 / 67	61	1.55	97 / 58	49
10.45	1.75	1.92	111 / 71	58	1.78	118 / 55	62	1.80	105 / 50	46
11.00	2.00	2.12	115 / 69	60	2.05	114 / 56	59	2.03	97 / 45	52
11.30	2.50	2.60	121 / 74	54	2.55	103 / 64	59	2.52	106 / 46	51
12.00	3.00	3.02	114 / 69	57	3.03	99 / 60	59	3.05	107 / 43	49
13.00	4.00	4.07	114 / 71	55	4.03	108 / 61	59	4.05	95 / 53	45
14.00	5.00	5.05	118 / 65	77	5.00	105 / 58	66	5.02	102 / 48	55
15.00	6.00	6.07	112 / 72	60	6.02	111 / 60	63	6.03	106 / 36	48
17.00	8.00	8.10	104 / 70	60	8.03	112 / 62	57	8.08	89 / 39	48
21.00	12.00	12.02	129 / 68	50	12.25	116 / 70	57	12.00	106 / 51	45
1.00	16.00	16.08	122 / 64	48	16.00	103 / 60	54	15.98	94 / 55	46
9.00	24.00	24.03	112 / 69	55	23.87	109 / 62	62	23.96	115 / 47	44

Volunteer number		4 DL			5 JB			6 MR		
Scheduled Time (hr)	*Scheme Time (hr)	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg) (b.p.m.)	Heart rate	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg) (b.p.m.)	Heart rate	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg) (b.p.m.)	Heart rate
8.55	-0.08	-0.12	110 / 65	64	-0.47	116 / 56	50	-0.62	112 / 63	64
9.10	0.17	0.20	98 / 56	72	0.20	92 / 45	60	0.27	85 / 40	88
9.20	0.33	0.37	99 / 45	77	0.37	91 / 49	60	0.48	103 / 45	86
9.30	0.50	0.55	95 / 38	85	0.55	93 / 56	63	0.65	97 / 39	75
9.35	0.58	0.62	101 / 46	82	0.62	93 / 37	56	0.75	101 / 51	80
9.40	0.67	0.72	96 / 50	81	0.72	103 / 42	68	0.87	104 / 57	74
9.50	0.83	0.87	99 / 47	70	0.87	93 / 50	57	1.08	102 / 55	79
10.00	1.00	1.03	100 / 64	75	1.03	94 / 52	49	1.23	104 / 57	70
10.10	1.17	1.20	103 / 62	76	1.18	93 / 45	54	1.38	132 / 45	68
10.20	1.33	1.38	103 / 55	65	1.35	91 / 46	58	1.58	105 / 60	76
10.30	1.50	1.55	101 / 52	69	1.53	98 / 54	54	1.75	108 / 54	68
10.45	1.75	1.78	97 / 54	67	1.78	96 / 55	49	1.98	110 / 55	66
11.00	2.00	2.03	103 / 59	66	2.05	98 / 55	51	2.22	107 / 59	71
11.30	2.50	2.57	101 / 57	71	2.52	84 / 45	52	2.55	109 / 63	67
12.00	3.00	2.95	100 / 64	67	3.07	100 / 51	52	3.03	103 / 62	77
13.00	4.00	4.00	101 / 59	65	4.02	96 / 45	54	4.03	110 / 62	71
14.00	5.00	5.02	105 / 65	75	5.02	99 / 47	60	5.05	105 / 61	79
15.00	6.00	6.02	104 / 67	70	6.02	104 / 49	58	5.90	106 / 64	75
17.00	8.00	8.02	107 / 57	68	7.97	98 / 53	58	8.12	105 / 61	64
21.00	12.00	12.03	118 / 76	65	11.98	110 / 58	59	12.10	110 / 62	65
1.00	16.00	15.92	110 / 58	66	16.08	89 / 49	51	16.23	118 / 73	67
9.00	24.00	23.58	120 / 59	70	23.78	102 / 55	60	23.52	117 / 51	73

Volunteer number		7 SG			8 RV			9 EL		
Scheduled Time (hr)	*Scheme Time (hr)	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg) (b.p.m.)	Heart rate	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg) (b.p.m.)	Heart rate	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg) (b.p.m.)	Heart rate
8.55	-0.08	-0.17	110 / 64	57	-0.08	136 / 72	68	-0.17	107 / 59	46
9.10	0.17	0.20	109 / 55	63	0.18	122 / 61	68	0.18	94 / 62	58
9.20	0.33	0.37	111 / 55	59	0.35	116 / 52	68	0.35	104 / 47	63
9.30	0.50	0.53	118 / 52	104	0.55	124 / 57	74	0.53	104 / 53	59
9.35	0.58	0.63	107 / 56	66	0.60	119 / 53	69	0.62	106 / 61	59
9.40	0.67	0.72	110 / 58	80	0.68	117 / 53	65	0.68	102 / 43	54
9.50	0.83	0.87	99 / 46	55	0.85	123 / 49	59	0.85	108 / 41	49
10.00	1.00	1.02	98 / 53	57	1.02	116 / 58	63	1.02	95 / 51	56
10.10	1.17	1.22	102 / 46	58	1.20	125 / 49	59	1.18	105 / 47	48
10.20	1.33	1.35	101 / 45	56	1.35	137 / 70	65	1.40	106 / 51	56
10.30	1.50	1.53	98 / 52	54	1.53	123 / 54	59	1.55	114 / 46	49
10.45	1.75	1.78	105 / 59	62	1.78	118 / 52	61	1.78	96 / 55	51
11.00	2.00	2.03	108 / 54	54	2.05	134 / 73	77	2.03	108 / 49	50
11.30	2.50	2.52	106 / 67	70	2.55	113 / 59	61	2.53	109 / 50	57
12.00	3.00	3.03	115 / 66	71	3.07	112 / 61	56	3.07	102 / 43	43
13.00	4.00	4.00	109 / 62	56	4.02	127 / 67	63	4.02	118 / 54	48
14.00	5.00	5.08	116 / 64	70	5.02	122 / 55	58	5.05	110 / 55	68
15.00	6.00	6.02	125 / 67	61	6.02	124 / 62	57	6.02	100 / 54	52
17.00	8.00	7.95	123 / 65	74	8.07	121 / 58	55	7.95	97 / 36	55
21.00	12.00	12.00	120 / 86	60	12.02	129 / 69	53	12.00	110 / 57	58
1.00	16.00	16.03	118 / 81	55	16.07	111 / 60	56	16.08	110 / 56	49
9.00	24.00	24.07	113 / 56	55	24.25	122 / 61	62	24.07	103 / 51	71

*= Scheme Time and Actual Time are expressed as a time-interval in relation to the time of sodium nitrite administration

Appendix 12a Table of Vital Signs

(continued)

Treatment B = Oral administration of 0.12 mmol NaNO₂ per mmol Hb

Volunteernumber		1 TN			2 MP			3 IS		
Scheduled Time (hr)	*Scheme Time (hr)	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg) (b.p.m.)	Heartrate	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg) (b.p.m.)	Heartrate	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg) (b.p.m.)	Heartrate
8.55	-0.08	-0.07	112 / 70	67	-0.75	118 / 66	61	-0.07	126 / 64	40
9.15	0.25	0.28	113 / 69	71	0.27	103 / 53	64	0.30	102 / 60	47
9.30	0.50	0.53	115 / 63	71	0.52	102 / 55	71	0.57	108 / 39	47
9.45	0.75	0.80	111 / 68	81	0.77	103 / 52	67	0.80	109 / 53	49
10.00	1.00	1.05	105 / 66	63	1.02	106 / 48	60	1.03	105 / 49	50
10.15	1.25	1.27	112 / 66	69	1.27	104 / 51	57	1.32	109 / 53	47
10.30	1.50	1.52	118 / 59	71	1.55	99 / 53	57	1.57	108 / 48	46
10.45	1.75	1.78	115 / 68	64	1.83	98 / 51	58	1.80	100 / 69	46
11.00	2.00	2.03	113 / 59	70	2.07	108 / 56	54	2.03	109 / 59	45
11.30	2.50	2.55	112 / 70	65	2.55	110 / 67	57	2.52	123 / 45	46
12.00	3.00	3.03	115 / 68	63	3.02	106 / 61	55	3.07	98 / 51	42
12.30	3.50	3.52	114 / 67	61	3.55	116 / 55	58	3.52	113 / 44	44
13.00	4.00	4.03	115 / 64	57	4.07	98 / 58	56	4.00	104 / 62	43
14.00	5.00	5.02	111 / 66	66	5.07	115 / 57	56	4.98	125 / 53	53
15.00	6.00	6.05	114 / 60	66	6.05	109 / 64	57	6.00	108 / 59	49
17.00	8.00	8.05	113 / 69	67	8.15	116 / 69	63	8.07	117 / 43	44
21.00	12.00	12.03	111 / 73	66	12.05	118 / 68	60	12.15	108 / 60	43
1.00	16.00	16.03	108 / 60	59	16.12	112 / 64	47	15.98	106 / 59	43
9.00	24.00	24.02	107 / 68	72	24.03	117 / 63	64	23.90	116 / 47	44

Volunteernumber		4 DL			5 JB			6 MR		
Scheduled Time (hr)	*Scheme Time (hr)	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg) (b.p.m.)	Heartrate	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg) (b.p.m.)	Heartrate	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg) (b.p.m.)	Heartrate
8.55	-0.08	-0.62	110 / 63	62	-0.63	98 / 60	53	-0.25	108 / 64	71
9.15	0.25	0.27	94 / 37	74	0.27	104 / 47	66	0.28	99 / 58	80
9.30	0.50	0.52	98 / 39	75	0.50	101 / 48	53	0.57	102 / 69	79
9.45	0.75	0.78	101 / 43	77	0.78	93 / 55	54	0.80	103 / 38	73
10.00	1.00	1.03	105 / 54	68	1.03	109 / 45	57	1.03	101 / 47	82
10.15	1.25	1.28	106 / 54	88	1.28	98 / 48	56	1.27	109 / 47	69
10.30	1.50	1.55	109 / 53	75	1.55	95 / 51	63	1.55	104 / 45	70
10.45	1.75	1.80	105 / 52	72	1.80	97 / 48	52	1.78	120 / 60	75
11.00	2.00	2.03	99 / 62	74	2.02	99 / 51	56	2.03	104 / 56	65
11.30	2.50	2.55	101 / 53	75	2.55	100 / 48	57	2.25	110 / 58	64
12.00	3.00	3.02	109 / 51	69	3.05	99 / 52	55	2.97	104 / 54	61
12.30	3.50	3.57	109 / 55	64	3.52	95 / 49	58	3.53	108 / 54	67
13.00	4.00	4.03	104 / 49	66	4.03	97 / 55	61	3.98	106 / 61	62
14.00	5.00	5.03	106 / 58	64	4.98	104 / 53	78	5.02	104 / 57	69
15.00	6.00	6.03	103 / 59	76	6.00	103 / 56	55	6.05	115 / 68	86
17.00	8.00	7.98	110 / 66	75	8.15	98 / 56	51	8.12	119 / 74	71
21.00	12.00	11.98	115 / 59	70	12.12	104 / 50	44	12.10	112 / 67	55
1.00	16.00	16.17	107 / 61	63	15.97	92 / 51	53	16.02	107 / 56	64
9.00	24.00	23.78	116 / 59	71	23.10	100 / 55	65	23.57	120 / 69	83

Volunteernumber		7 SG			8 RV			9 EL		
Scheduled Time (hr)	*Scheme Time (hr)	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg) (b.p.m.)	Heartrate	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg) (b.p.m.)	Heartrate	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg) (b.p.m.)	Heartrate
8.55	-0.08	-0.10	122 / 65	58	-0.08	120 / 53	52	-0.05	117 / 64	48
9.15	0.25	0.32	109 / 68	75	0.27	108 / 48	60	0.28	107 / 56	63
9.30	0.50	0.55	98 / 56	79	0.50	108 / 50	52	0.55	99 / 44	64
9.45	0.75	0.83	102 / 52	68	0.75	108 / 46	52	0.82	111 / 51	53
10.00	1.00	1.05	111 / 54	71	1.00	103 / 46	52	1.00	109 / 50	50
10.15	1.25	1.37	109 / 60	68	1.25	113 / 46	51	1.28	99 / 55	53
10.30	1.50	1.55	113 / 53	55	1.50	114 / 46	52	1.53	107 / 53	51
10.45	1.75	1.80	131 / 50	57	1.77	112 / 52	52	1.78	105 / 58	56
11.00	2.00	2.05	111 / 70	61	2.02	122 / 56	59	2.02	104 / 47	46
11.30	2.50	2.58	108 / 50	62	2.53	125 / 67	74	2.55	102 / 60	52
12.00	3.00	3.03	109 / 48	54	3.02	109 / 60	52	3.03	106 / 58	47
12.30	3.50	3.55	105 / 59	49	3.53	114 / 59	51	3.52	109 / 46	43
13.00	4.00	4.03	108 / 62	61	3.98	111 / 61	51	4.02	109 / 54	45
14.00	5.00	5.05	111 / 64	67	5.00	115 / 55	58	5.02	118 / 52	52
15.00	6.00	6.12	117 / 66	64	5.98	106 / 56	56	6.07	115 / 46	54
17.00	8.00	8.02	122 / 74	74	7.92	108 / 55	53	8.03	119 / 68	47
21.00	12.00	12.02	116 / 70	54	11.93	121 / 52	55	12.03	119 / 65	46
1.00	16.00	16.08	99 / 49	54	16.07	116 / 58	58	16.13	111 / 45	40
9.00	24.00	24.05	120 / 71	77	24.03	120 / 64	49	24.10	119 / 48	44

*= Scheme Time and Actual Time are expressed as a time-interval in relation to the time of sodium nitrite administration

Appendix 12a Table of Vital Signs(continued) Treatment C = Oral administration of 0.06 mmol NaNO₂ per mmol Hb

Volunteernumber		1 TN			2 MP			3 IS		
Scheduled Time (hr)	*Scheme Time (hr)	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg)	Heartrate (b.p.m.)	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg)	Heartrate (b.p.m.)	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg)	Heartrate (b.p.m.)
8.55	-0.08	-0.03	109 / 70	52	-0.20	116 / 59	56	-0.88	113 / 73	52
9.15	0.25	0.28	111 / 61	60	0.20	95 / 57	65	0.22	112 / 42	49
9.30	0.50	0.53	107 / 63	63	0.57	104 / 59	58	0.50	103 / 47	58
9.45	0.75	0.80	116 / 63	61	0.77	93 / 65	58	0.72	111 / 50	48
10.00	1.00	1.03	107 / 67	57	1.00	99 / 63	67	0.97	131 / 51	48
10.15	1.25	1.28	109 / 69	56	1.27	100 / 57	58	1.25	106 / 67	45
10.30	1.50	1.55	109 / 69	62	1.52	100 / 58	63	1.52	103 / 58	44
10.45	1.75	1.82	115 / 65	63	1.75	107 / 59	60	1.75	106 / 63	44
11.00	2.00	2.03	112 / 67	65	2.00	98 / 50	57	1.98	112 / 52	49
11.30	2.50	2.53	108 / 70	59	2.50	102 / 59	58	2.45	106 / 65	44
12.00	3.00	3.05	117 / 66	63	3.07	104 / 52	56	2.97	101 / 65	41
12.30	3.50	3.55	115 / 69	65	3.50	100 / 62	57	3.47	106 / 65	43
13.00	4.00	4.02	112 / 72	65	4.00	106 / 56	55	3.97	105 / 62	41
14.00	5.00	5.02	112 / 66	68	5.02	117 / 65	63	5.00	112 / 59	51
15.00	6.00	6.02	111 / 67	68	6.02	112 / 71	64	5.97	116 / 54	47
17.00	8.00	8.02	105 / 66	59	8.02	119 / 65	61	8.12	110 / 70	48
21.00	12.00	12.18	121 / 74	54	12.00	115 / 71	58	12.05	111 / 70	45
1.00	16.00	16.00	112 / 62	58	15.95	122 / 66	62	16.03	110 / 65	41
9.00	24.00	23.88	116 / 69	66	23.93	117 / 68	63	23.97	119 / 60	42

Volunteernumber		4 DL			5 JB			6 MR		
Scheduled Time (hr)	*Scheme Time (hr)	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg)	Heartrate (b.p.m.)	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg)	Heartrate (b.p.m.)	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg)	Heartrate (b.p.m.)
8.55	-0.08	-0.60	113 / 60	59	-0.40	104 / 48	51	-0.55	113 / 65	71
9.15	0.25	0.28	102 / 50	68	0.27	104 / 43	63	0.27	108 / 56	70
9.30	0.50	-	- / -	-	0.53	102 / 46	58	0.57	113 / 60	71
9.45	0.75	0.80	112 / 61	71	0.78	100 / 46	57	0.78	106 / 54	63
10.00	1.00	1.03	99 / 56	69	1.03	100 / 51	66	1.05	107 / 54	62
10.15	1.25	1.32	108 / 50	73	1.32	100 / 47	58	1.27	107 / 54	62
10.30	1.50	1.55	107 / 56	74	1.53	108 / 51	58	1.53	105 / 63	64
10.45	1.75	1.85	103 / 56	69	1.82	105 / 54	59	1.75	110 / 66	64
11.00	2.00	2.07	104 / 53	64	2.03	103 / 54	50	2.00	107 / 58	61
11.30	2.50	2.57	113 / 66	72	2.28	106 / 66	55	2.52	114 / 54	66
12.00	3.00	3.05	113 / 56	63	2.97	132 / 54	59	3.02	116 / 60	65
12.30	3.50	3.57	113 / 55	64	3.53	102 / 47	50	3.52	121 / 70	64
13.00	4.00	4.07	117 / 64	67	4.02	98 / 53	55	3.97	115 / 65	63
14.00	5.00	5.03	128 / 66	73	5.03	107 / 47	74	5.02	117 / 62	76
15.00	6.00	6.03	111 / 50	71	6.05	103 / 52	51	6.03	112 / 63	76
17.00	8.00	8.13	115 / 52	65	8.08	106 / 51	51	8.05	104 / 66	63
21.00	12.00	12.13	113 / 64	72	12.08	95 / 42	46	11.93	117 / 65	67
1.00	16.00	15.97	116 / 73	60	15.92	106 / 49	54	16.18	102 / 57	64
9.00	24.00	23.32	124 / 60	65	23.58	106 / 58	53	23.78	120 / 67	65

Volunteernumber		7 SG			8 RV			9 EL		
Scheduled Time (hr)	*Scheme Time (hr)	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg)	Heartrate (b.p.m.)	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg)	Heartrate (b.p.m.)	*Actual Time (hr)	Blood Pressure Systole / Diastole (mmHg) (mmHg)	Heartrate (b.p.m.)
8.55	-0.08	-0.10	122 / 62	51	-0.02	121 / 63	58	-0.27	111 / 44	43
9.15	0.25	0.37	121 / 54	58	0.28	117 / 57	77	0.28	106 / 37	54
9.30	0.50	0.55	136 / 55	56	0.57	110 / 45	65	0.53	111 / 41	59
9.45	0.75	0.78	104 / 59	74	0.82	111 / 55	59	0.78	133 / 44	46
10.00	1.00	1.05	120 / 70	68	1.03	107 / 44	55	1.03	120 / 47	59
10.15	1.25	1.30	118 / 58	59	1.30	117 / 42	56	1.30	86 / 37	48
10.30	1.50	1.57	111 / 55	55	1.55	113 / 43	55	1.53	108 / 45	53
10.45	1.75	1.82	114 / 64	52	1.78	108 / 48	55	1.83	101 / 36	45
11.00	2.00	2.05	119 / 70	68	2.05	108 / 53	56	2.05	103 / 46	53
11.30	2.50	2.55	123 / 72	59	2.57	111 / 61	56	2.53	108 / 47	46
12.00	3.00	3.05	113 / 68	60	3.05	108 / 54	60	3.02	104 / 49	51
12.30	3.50	3.52	128 / 64	60	3.55	107 / 55	53	3.57	107 / 44	46
13.00	4.00	4.03	111 / 65	62	4.05	133 / 57	59	4.02	113 / 46	48
14.00	5.00	5.03	119 / 60	61	5.05	128 / 67	65	5.02	120 / 55	78
15.00	6.00	6.08	120 / 53	49	6.10	113 / 64	58	6.08	118 / 55	68
17.00	8.00	8.05	125 / 65	68	8.03	111 / 66	61	8.05	105 / 82	70
21.00	12.00	11.98	121 / 79	61	12.05	122 / 69	56	12.05	118 / 72	82
1.00	16.00	16.08	122 / 69	68	16.10	114 / 55	54	16.00	109 / 45	45
9.00	24.00	24.18	127 / 64	77	24.08	126 / 59	55	24.02	98 / 39	46

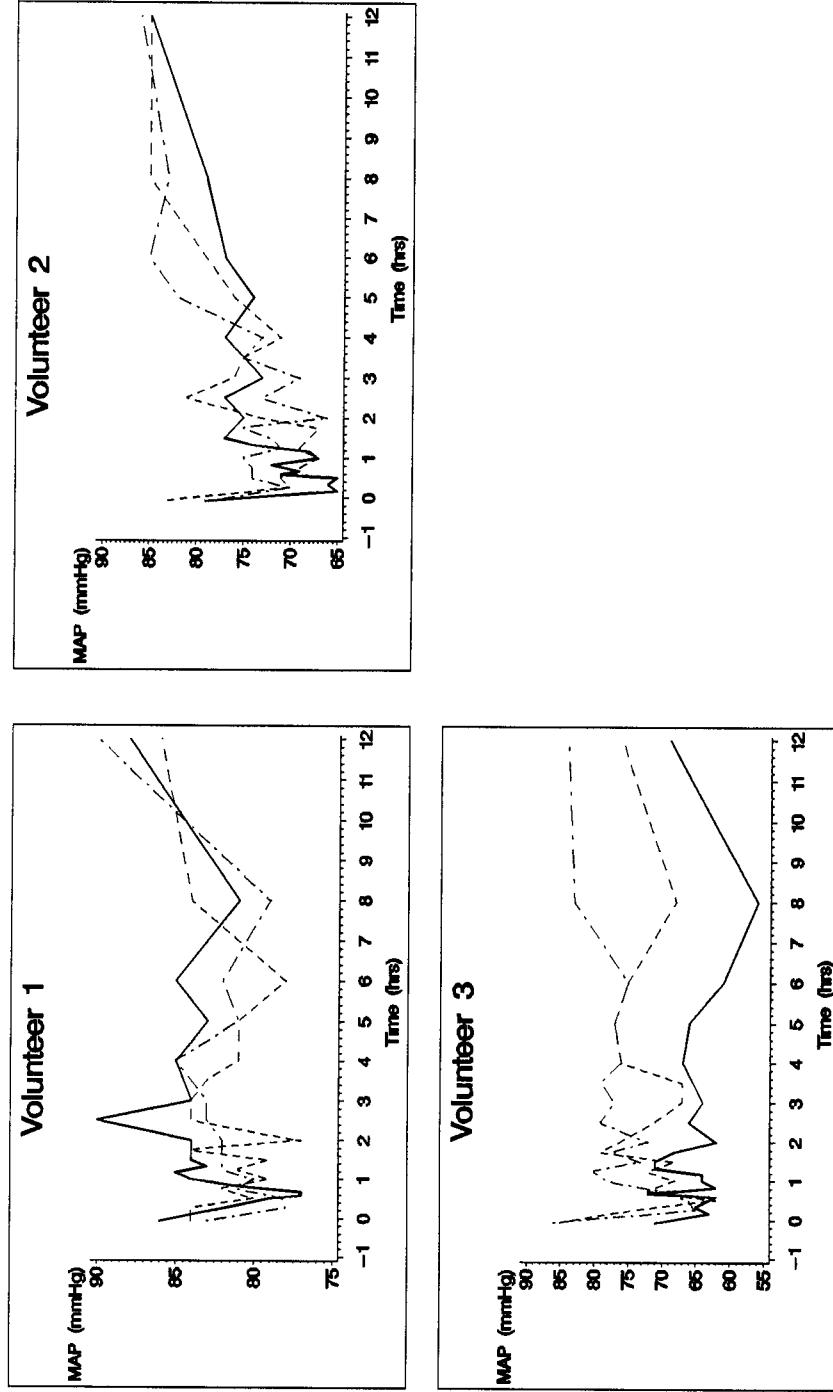
*= Scheme Time and Actual Time are expressed as a time-interval in relation to the time of sodium nitrite administration

Appendix 12b Mean Arterial Pressure as observed after single dose administration of sodium nitrite to adult volunteers

(—) = Treatment A (intravenous administration of 0.12 mmol sodium nitrite per mmol Hb)

(---) = Treatment B (oral administration of 0.12 mmol sodium nitrite per mmol Hb)

(-·-) = Treatment C (oral administration of 0.06 mmol sodium nitrite per mmol Hb)

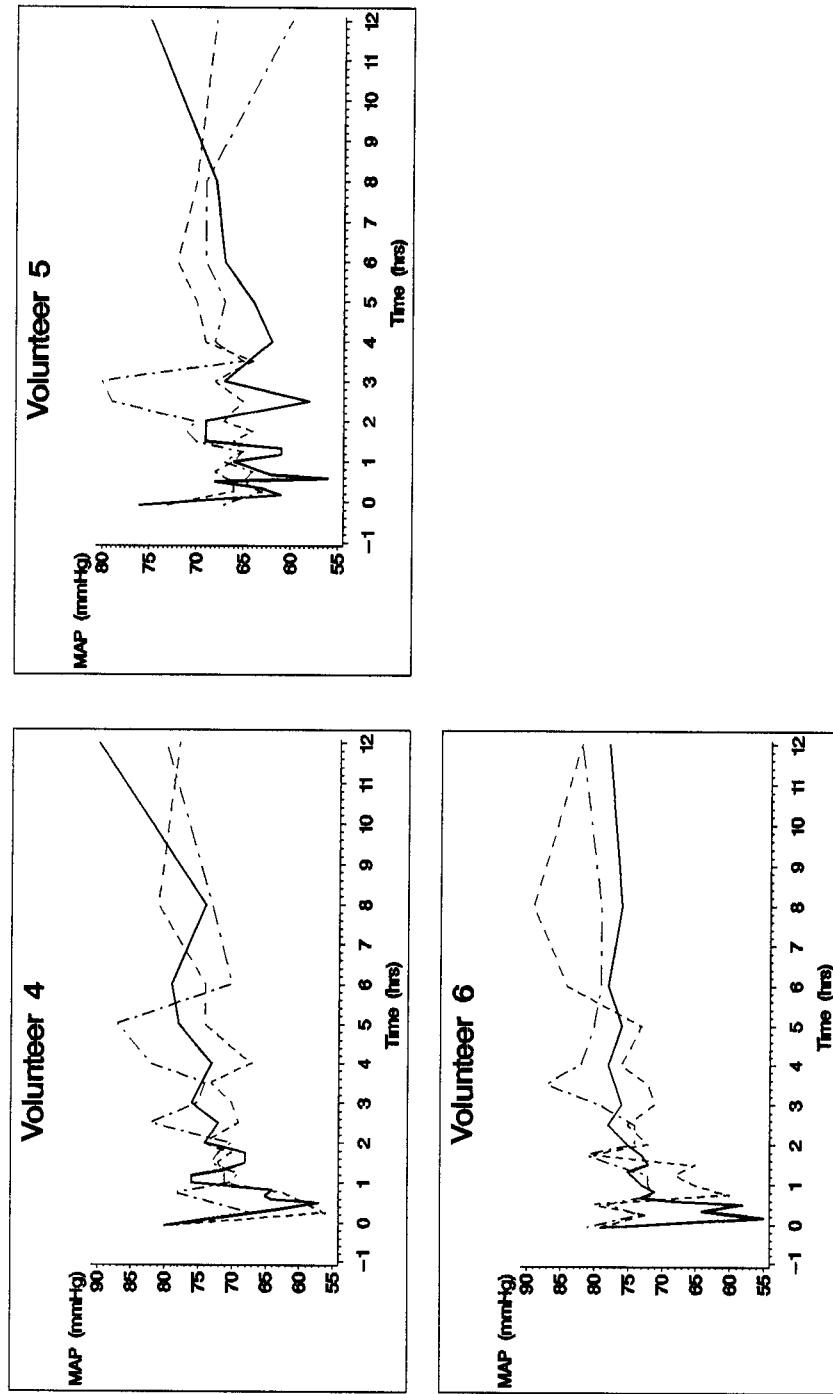


Appendix 12b Mean Arterial Pressure as observed after single dose administration of sodium nitrite to adult volunteers
(continued)

(—) = Treatment A (intravenous administration of 0.12 mmol sodium nitrite per mmol Hb)

(---) = Treatment B (oral administration of 0.12 mmol sodium nitrite per mmol Hb)

(- - -) = Treatment C (oral administration of 0.06 mmol sodium nitrite per mmol Hb)

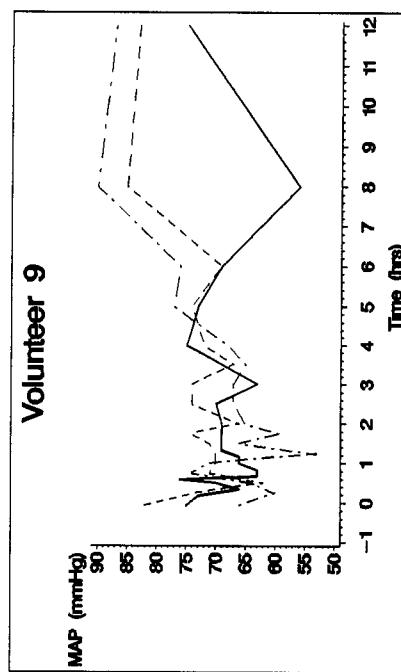
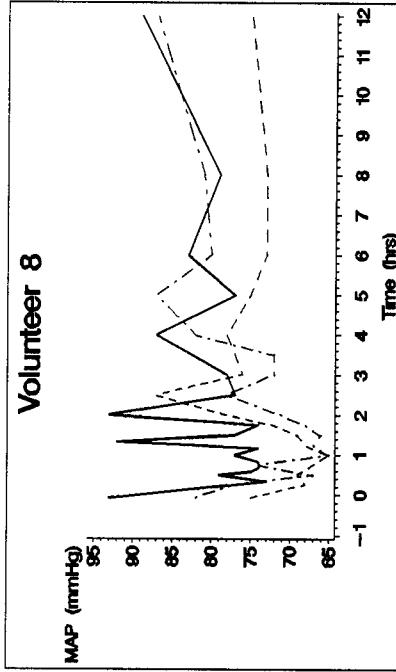
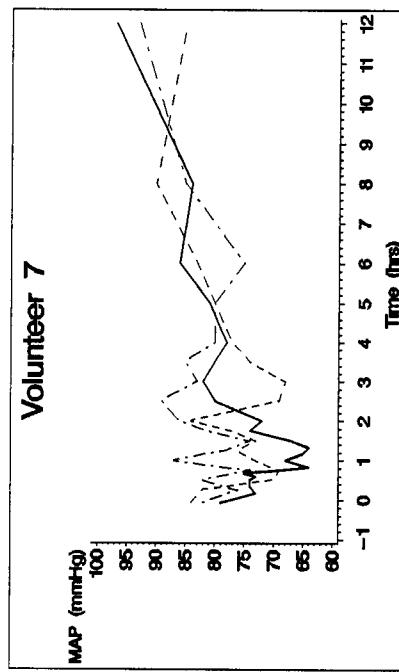


Appendix 12b Mean Arterial Pressure as observed after single dose administration of sodium nitrite to adult volunteers
(continued)

(—) = Treatment A (intravenous administration of 0.12 mmol sodium nitrite per mmol Hb)

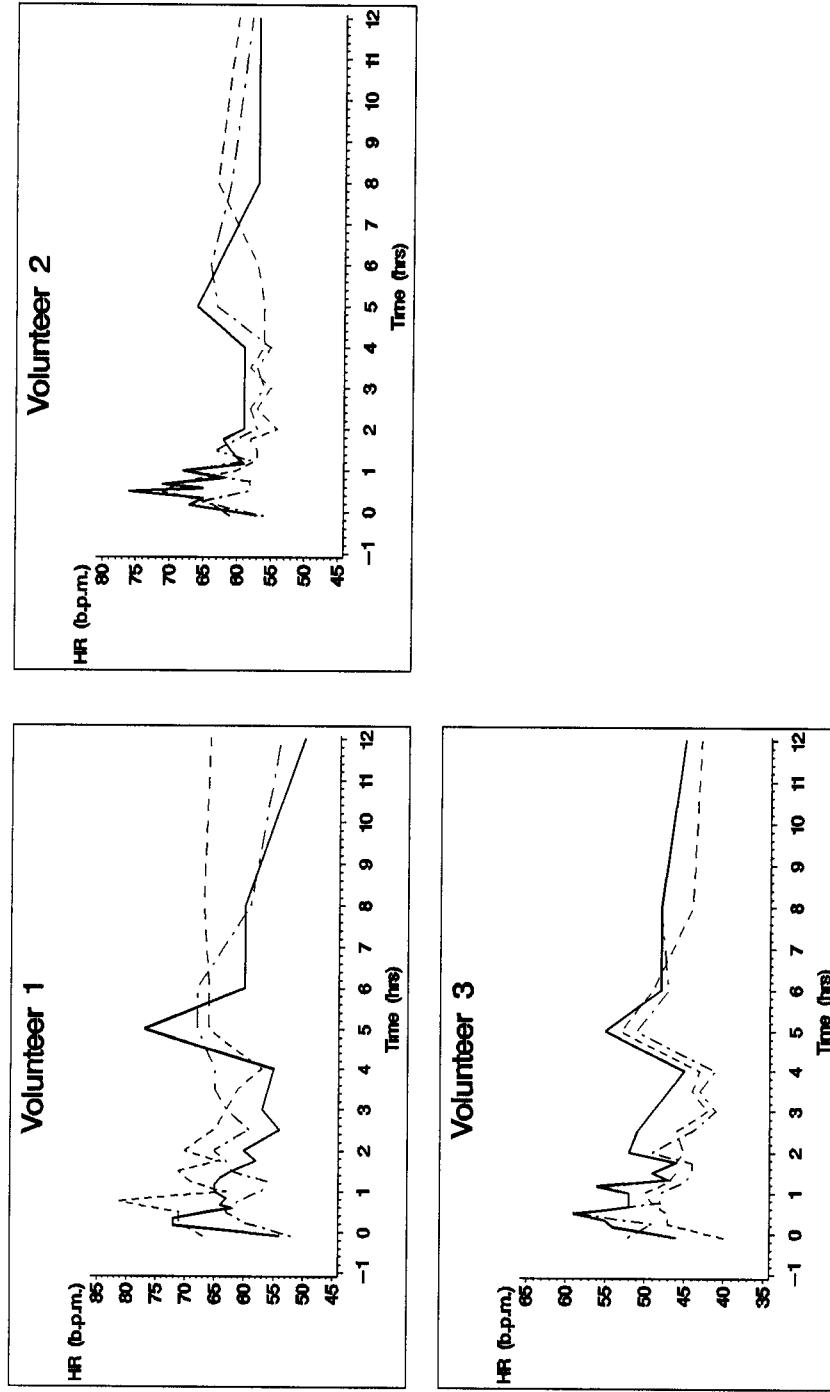
(---) = Treatment B (oral administration of 0.12 mmol sodium nitrite per mmol Hb)

(—·—) = Treatment C (oral administration of 0.06 mmol sodium nitrite per mmol Hb)



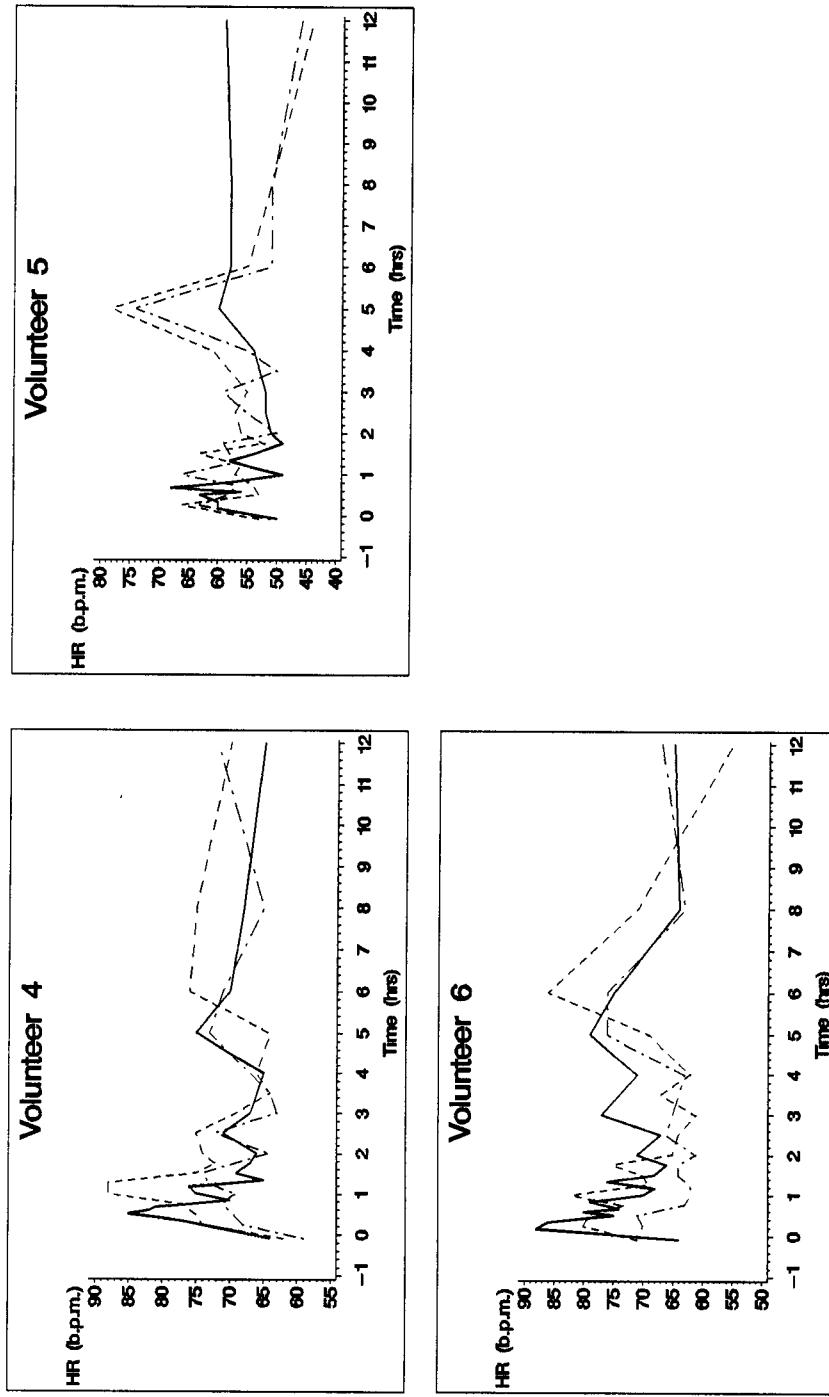
Appendix I2c Heart Rate as observed after single dose administration of sodium nitrite to adult volunteers

- (—) = Treatment A (intravenous administration of 0.12 mmol sodium nitrite per mmol Hb)
(---) = Treatment B (oral administration of 0.12 mmol sodium nitrite per mmol Hb)
(- - -) = Treatment C (oral administration of 0.06 mmol sodium nitrite per mmol Hb)



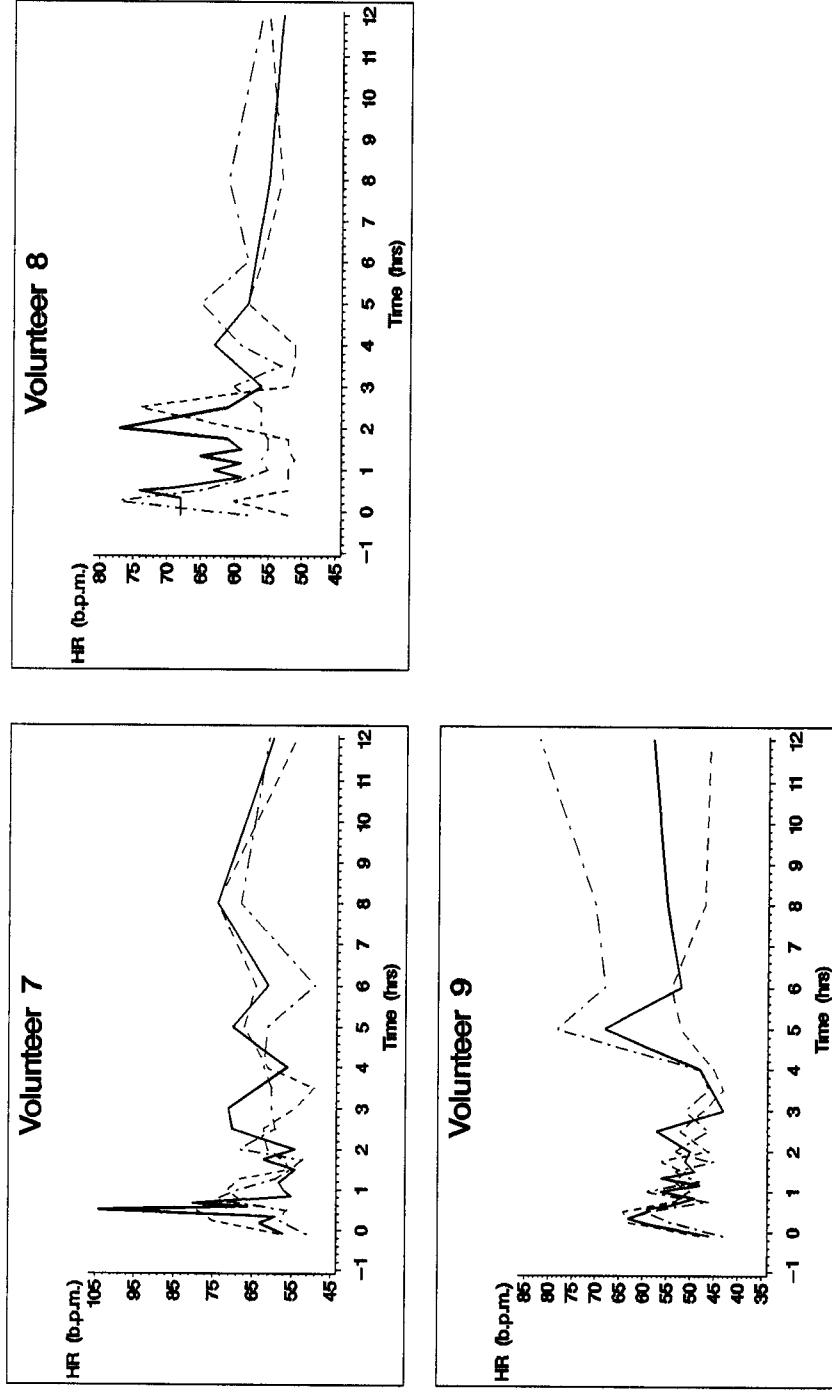
Appendix 12c Heart Rate as observed after single dose administration of sodium nitrite to adult volunteers
(continued)

- (—) = Treatment A (intravenous administration of 0.12 mmol sodium nitrite per mmol Hb)
(---) = Treatment B (oral administration of 0.12 mmol sodium nitrite per mmol Hb)
(-·-) = Treatment C (oral administration of 0.06 mmol sodium nitrite per mmol Hb)



Appendix 12c Heart Rate as observed after single dose administration of sodium nitrite to adult volunteers
(continued)

- (—) = Treatment A (intravenous administration of 0.12 mmol sodium nitrite per mmol Hb)
(---) = Treatment B (oral administration of 0.12 mmol sodium nitrite per mmol Hb)
(-·-) = Treatment C (oral administration of 0.06 mmol sodium nitrite per mmol Hb)



Appendix 13a Table of Hemoglobin concentrations

Treatment A = Intravenous administration of 0.12 mmol NaNO₂ per mmol Hb to adult volunteers

Volunteer number	1 TN		2 MP		3 IS		4 DL		5 JB		6 MR		7 SG		8 RV		9 EL		
	Scheduled Time (hr)	Scheme Time (hr)	Actual Time (hr)	Hb (mmol/l)															
8.00	-1.00	-2.25	8.31	-1.58	9.18	-1.50	8.49	-1.85	8.06	-2.00	7.81	-1.48	8.56	-1.17	8.06	-1.50	9.30	-1.22	7.94
8.55	-0.08	-1.52	8.43	-0.17	9.24	-0.33	8.00	-0.17	7.94	-0.42	7.94	-0.63	7.69	-0.12	7.94	-0.58	8.99	-0.20	7.38
9.10	0.17	0.22 *	8.56	0.17	9.11	0.17	8.12	0.17	7.81	0.17	7.69	0.20	7.63	0.18	7.87	0.17	9.11	0.17	7.38
9.20	0.33	0.30 *	8.49	0.33	9.18	0.25	8.00	0.33	7.87	0.33	7.63	0.37	7.38	0.33	8.99	0.33	8.99	0.33	7.50
9.30	0.50	0.53	8.43	0.52	9.11	0.50	8.06	0.52	7.81	0.53	7.63	0.53	7.25	0.50	7.87	0.50	8.93	0.50	7.44
9.35	0.58	0.63	8.37	0.60	9.11	0.62	7.94	0.60	7.81	0.60	7.63	0.73	7.25	0.60	8.87	0.58	8.97	0.58	7.38
9.40	0.67	0.78	8.56	0.67	9.11	0.72	8.00	0.68	7.81	0.68	7.63	0.83	7.38	0.67	8.00	0.67	8.93	0.67	7.25
9.50	0.83	0.88	8.43	0.83	9.18	0.87	7.94	0.83	7.75	0.83	7.55	1.00	7.32	0.83	7.87	0.83	8.93	0.83	7.38
10.00	1.00	1.05	8.49	1.00	9.05	1.00	7.81	1.02	7.75	1.00	7.63	1.17	7.25	1.00	7.87	1.00	8.99	1.00	7.38
10.10	1.17	1.22	8.25	1.17	9.05	1.20	8.00	1.17	7.63	1.17	7.63	1.33	7.19	1.18	7.81	1.17	8.87	1.17	7.38
10.20	1.33	1.38	8.18	1.35	8.93	1.33	7.94	1.35	7.56	1.33	7.56	1.53	7.25	1.33	7.69	1.33	8.87	1.33	7.38
10.30	1.50	1.58	8.12	1.50	8.93	1.52	7.94	1.50	7.56	1.50	7.63	1.70	7.25	1.50	7.69	1.50	8.93	1.50 *	7.63
10.45	1.75	1.88	8.18	1.75	8.93	1.75	8.00	1.75	7.56	1.75	7.63	1.92	7.50	1.75	7.94	1.75	8.93	1.75	7.32
11.00	2.00	2.05	8.31	2.00	8.87	2.00	7.94	2.02	7.50	2.02	7.56	2.17	7.50	2.00	7.81	2.00	9.05	2.00	7.25
11.30	2.50	2.55	8.25	2.50	8.99	2.50	8.00	2.53	7.50	2.50	7.56	2.50	7.32	2.50	7.87	2.50	8.93	2.50	7.32
12.00	3.00	3.00	8.12	3.00	9.18	3.00	7.94	2.93	7.50	3.00	7.63	3.00	7.19	3.00	7.81	3.02	8.93	3.00	7.32
13.00	4.02	4.25	4.00	4.00	9.05	4.00	7.81	4.00	7.50	4.00	7.69	4.00	7.28	4.00	7.81	4.00	8.80	4.00	7.50
14.00	5.00	5.43	4.98	4.92	5.00	5.00	8.31	5.00	7.87	5.00	7.87	5.05	5.05	5.00	8.06	5.03	9.30	5.03	7.94
15.00	6.00	6.05	8.49	6.00	9.49	6.00	8.31	6.00	7.63	6.00	7.75	5.92	7.63	5.98	8.25	6.00	8.99	5.98	7.69
17.00	8.00	8.37	8.00	9.30	8.05	7.81	8.00	7.69	8.00	7.75	8.10	8.00	7.98	8.00	8.03	9.18	7.98	7.32	
21.00	12.00	11.98	8.12	12.13	8.93	12.00	7.75	12.00	7.69	12.00	7.50	12.03	7.75	12.00	7.18	12.00	7.69	12.03	
1.00	16.00	16.00	7.94	16.00	8.62	15.92	7.56	16.08	7.69	16.17	7.25	16.00	7.69	16.00	8.68	16.03	7.13	16.03	
9.00	24.00	23.92 *	8.56	23.83	9.05	23.95 *	8.49	23.55 *	8.25	23.75 *	8.62	23.45	8.06	24.03 *	8.37	24.00	9.36	24.02	7.50

The Scheme Time and Actual Time are expressed as an interval in hours passing by between the time of measurement and the time that sodium nitrite was administered

* = Blood sampling under tourniquet pressure

+ = Blood sampling probably under tourniquet pressure

**Appendix 13a Table of Hemoglobin concentrations
(continued)**

Treatment B = Oral administration of 0.12 mmol NaNO₂ per mmol Hb to adult volunteers

Volunteer number	1 TN		2 MP		3 IS		4 DL		5 JB		6 MR		7 SG		8 RV		9 EL	
	Scheme Time (hr)	Actual Time (hr)	Hb (mmol/l)	Actual Time (hr)														
8.00	-1.00	-1.25	8.18	-1.55	9.36	-0.83 *	8.31	-1.08	7.94	-1.78 *	8.37	-0.92	7.87	-1.17 *	8.06	-1.00 *	8.56	ND
8.55	-0.08	-0.03	8.18	-0.55	9.42	-0.08 *	8.43	-0.77	7.75	-0.67 *	8.31	-0.38	7.63	-0.08 *	8.31	-0.17	8.56	-0.42 *
9.15	0.25	0.25	8.12	0.25	9.24	0.25 *	8.43	0.25	7.75	0.25 *	8.25	0.25	7.50	0.25 *	8.12	0.25	8.25	0.25
9.30	0.50	0.50 *	8.06	0.50	9.36	0.50 *	8.49	0.48	7.63	0.47 *	8.25	0.50	7.44	0.50	8.06	0.50	8.18	0.50
9.45	0.75	0.75	8.00	0.75	9.24	0.75 *	8.74	0.80	7.50	0.75	8.18	0.75	7.50	0.78	7.94	0.75	8.31	0.75
10.00	1.00	1.00	8.06	1.00	9.24	1.00 *	8.37	1.00	7.56	1.00	8.12	1.00	7.50	1.02	7.81	1.00	8.31	0.98
10.15	1.25	1.25	8.06	1.25	9.18	1.25	8.12	1.25	7.56	1.25	8.06	1.25	7.44	1.30 *	7.81	1.25	8.25	1.25
10.30	1.50	1.50	8.00	1.50	9.24	1.50 *	8.37	1.50	7.56	1.50	8.00	1.50	7.44	1.53 *	8.00	1.50	8.49	1.50
10.45	1.75	1.75	8.00	1.80	9.11	1.75	8.18	1.75	7.56	1.75	7.94	1.75	7.56	1.60 *	8.06	1.75	8.43	1.75
11.00	2.00	2.00	8.00	2.03	9.11	2.00	8.18	2.00	7.44	2.00	7.81	2.00	7.50	2.02	7.75	2.00	8.43	2.00
11.30	2.50	2.50	7.94	2.52	9.25	2.50	7.44	2.50	7.44	2.50	7.44	2.50	7.50	2.23	7.81	2.50	8.49	2.50
12.00	3.00	3.00	7.94	3.00	9.05	3.00	8.18	3.00	7.44	3.00 *	8.00	2.92	7.32	3.00	7.87	3.00	8.37	3.00
12.30	3.50	3.50	7.87	3.52	9.11	3.50	8.18	3.52	7.38	3.50	7.87	3.50	7.25	3.50	7.94	3.50	8.43	3.50
13.00	4.00	4.00	7.94	4.03	9.11	4.00	8.06	4.00	7.38	4.00	8.06	3.98	7.44	4.00 *	8.06	3.98	8.18	4.00
14.00	5.00	5.00	8.18	5.02	9.55	5.00	8.68	5.00	7.38	4.97	8.12	5.00	7.69	5.02 *	8.56	4.98	8.62	5.00
15.00	6.00	6.02	8.31	6.02	9.42	6.00	8.56	6.00	7.50	5.98	8.25	6.00	8.06	6.03	8.56	6.05	8.98	6.05
17.00	8.00	8.00	8.13	8.42	8.00 *	8.80	8.00	7.94	8.12	8.25	8.08	8.12	8.00	8.31	7.95	8.31	8.00	7.81
21.00	12.00	12.00	7.81	11.98	9.36	12.00	8.56	12.00	7.56	12.10	7.87	12.08	8.00	12.00	8.18	11.98	8.43	12.00
1.00	16.00	16.00	7.94	16.00	9.30	15.98	7.81	16.08	7.63	15.92	7.81	16.02	7.38	16.00	7.75	16.07	8.18	16.08
9.00	24.00	24.00	8.37	23.93 *	9.80	23.83 *	8.31	23.75 *	8.18	23.08 *	8.56	23.55 *	8.06	24.00 *	8.31	24.00 *	8.74	24.05

The Scheme Time and Actual Time are expressed as an interval in hours passing by between the time of measurement and the time that sodium nitrite was administered
* = Blood sampling under tourniquet pressure

**Appendix 13a Table of Hemoglobin concentrations
(continued)**

Treatment C = Oral administration of 0.06 mmol NaNO₂ per mmol Hb to adult volunteers

Volunteer number	1 TN		2 MP		3 IS		4 DL		5 JB		6 MR		7 SG		8 RV		9 EL		
	Scheme Time (hr)	Actual Time (hr)	Hb (mmol/l)																
8.00	-1.00	-1.55	7.94	-1.58	9.30	-3.00	8.37	-1.87	8.31	-1.25	7.87	-0.83	8.25	-1.67	8.18	-1.58 *	9.11	-1.08	7.25
8.55	-0.08	-0.08	8.12	-0.12	8.99	-1.38	8.06	-0.72	8.31	-0.42	7.94	-0.25	7.75	-0.75	8.06	-0.08 *	9.24	-0.08	7.19
9.15	0.25	0.25	8.00	0.17	8.93	0.25	7.94	0.25	8.18	0.25	7.87	0.25	7.69	0.30 *	8.18	0.25	8.87	0.25	7.07
9.30	0.50	0.50	7.94	0.53 *	8.99	0.50	8.00	0.50	8.12	0.50	7.94	0.50	7.56	0.50	7.94	0.50	8.68	0.50	7.07
9.45	0.75	0.75	8.00	0.75	8.87	0.75	7.94	0.77	8.00	0.77	7.94	0.75	7.44	0.75	7.87	0.75	8.87	0.75	7.01
10.00	1.00	1.00	8.00	1.00	8.74	1.00	7.81	1.00	7.94	1.00	7.87	1.00	7.56	1.00	7.87	1.00	8.68	1.00	7.07
10.15	1.25	1.25	7.87	1.25	8.68	1.25	7.94	1.27	8.00	1.25	7.81	1.25	7.50	1.25	7.87	1.25	8.68	1.25	7.07
10.30	1.50	1.52	7.75	1.50	8.74	1.50	7.81	1.50	7.94	1.50	7.81	1.50	7.75	1.50	7.94	1.50	8.56	1.50	7.01
10.45	1.75	1.78	7.81	1.73	8.80	1.77	7.87	1.77	8.06	1.75	7.81	1.75	7.75	1.75	8.00	1.75	8.62	1.75	7.07
11.00	2.00	2.00	7.81	1.98	8.74	2.02	7.87	2.00	7.87	2.00	7.63	1.98	7.50	2.00	7.87	2.00	8.49	2.00	6.94
11.30	2.50	2.50	7.87	2.50	8.87	2.50	7.94	2.52	7.87	2.25	7.69	2.50	7.44	2.50	8.06	2.52	8.43	2.50	6.82
12.00	3.00	3.00	7.81	3.00	8.80	3.00	8.00	3.00	7.94	2.92	7.63	3.00	7.56	3.03	7.94	3.00	8.49	3.00	6.94
12.30	3.50	3.50	7.75	3.50	8.74	3.50	7.87	3.50	8.00	3.50	7.63	3.48	7.50	3.50	7.94	3.50	8.62	3.50	6.88
13.00	4.00	4.00	7.81	4.00	8.93	4.00	7.94	4.00	7.81	4.00	7.75	4.00	7.69	4.00	8.00	4.00	8.56	4.00	6.94
14.00	5.00	5.00	8.18	5.00	9.36	5.00	8.37	5.00	7.87	5.00	7.75	4.98	8.00	5.00	8.25	5.00	8.93	5.00	7.69
15.00	6.00	6.00	8.25	6.00	9.42	6.00	8.31	6.00	8.06	6.02	7.87	6.00	8.00	6.03	8.31	6.00	8.93	6.05	7.19
17.00	8.00	8.00	8.18	8.00	9.36	8.08	8.25	8.12	8.00	8.12	8.00	8.06	8.02	8.31	8.00	8.93	8.00	7.44	
21.00	12.00	12.17	8.12	12.00	8.93	12.00	8.43	12.05	7.94	12.03	11.97	7.63	12.00	8.12	8.74	12.00	7.38	12.00	7.07
1.00	16.00	16.03	7.87	15.97	8.87	16.00	7.81	15.92	7.87	16.08	7.94	16.12	7.63	16.00	7.81	16.08	8.25	15.98	6.94
9.00	24.00	23.83 *	8.43	23.92	9.42	24.00	8.18	23.25	8.25	23.57 *	8.43	23.75 *	8.49	24.08 *	8.49	24.03	9.11	23.95 *	7.56

The Scheme Time and Actual Time are expressed as an interval in hours passing by between the time of measurement and the time that sodium nitrite was administered

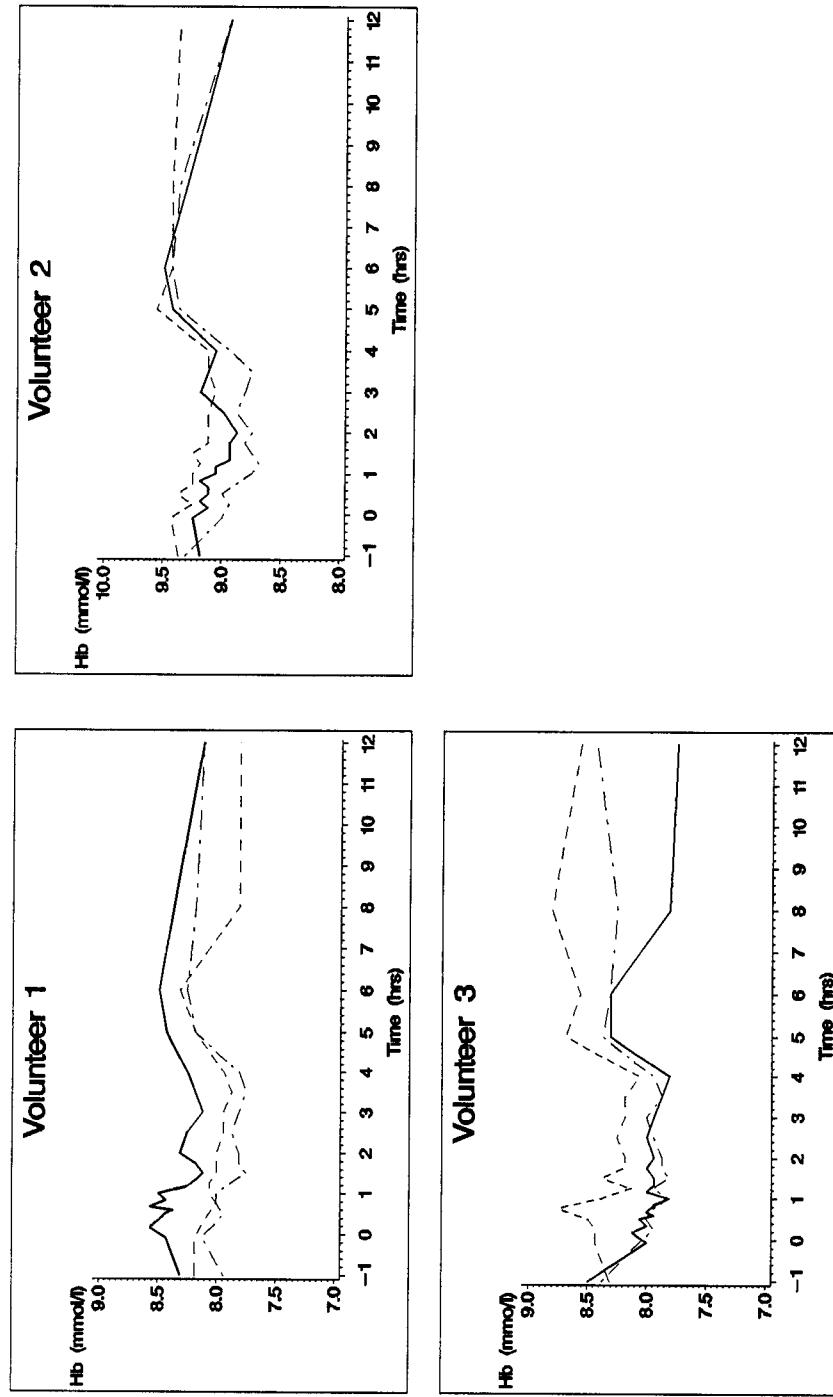
* = Blood sampling under tourniquet pressure

Appendix 13b Hemoglobin concentration as observed after single dose administration of sodium nitrite to adult volunteers

(—) = Treatment A (intravenous administration of 0.12 mmol sodium nitrite per mmol Hb)

(---) = Treatment B (oral administration of 0.12 mmol sodium nitrite per mmol Hb)

(-·-) = Treatment C (oral administration of 0.06 mmol sodium nitrite per mmol Hb)

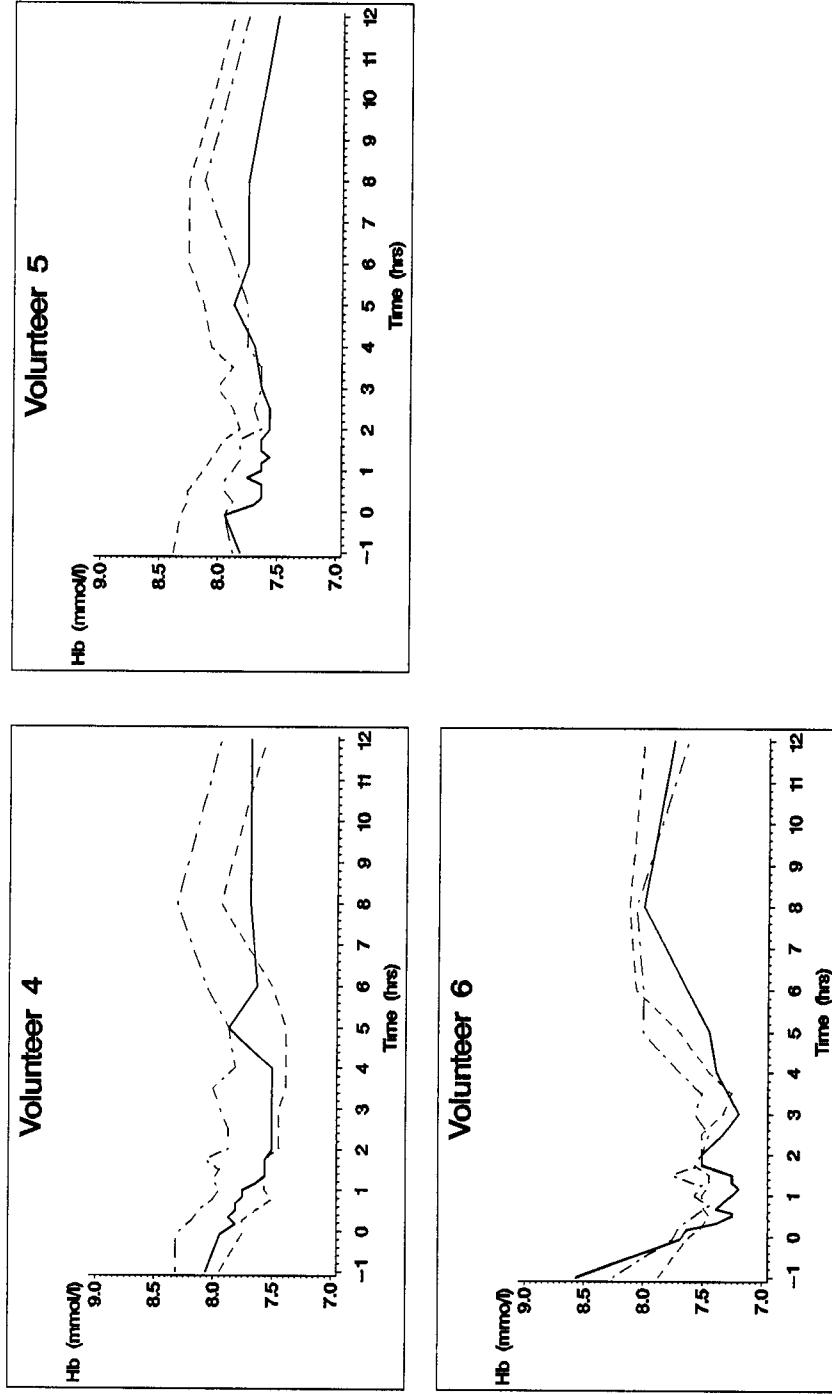


Appendix 13b Hemoglobin concentration as observed after single dose administration of sodium nitrite to adult volunteers
(continued)

(—) = Treatment A (intravenous administration of 0.12 mmol sodium nitrite per mmol Hb)

(- - -) = Treatment B (oral administration of 0.12 mmol sodium nitrite per mmol Hb)

(- · -) = Treatment C (oral administration of 0.06 mmol sodium nitrite per mmol Hb)

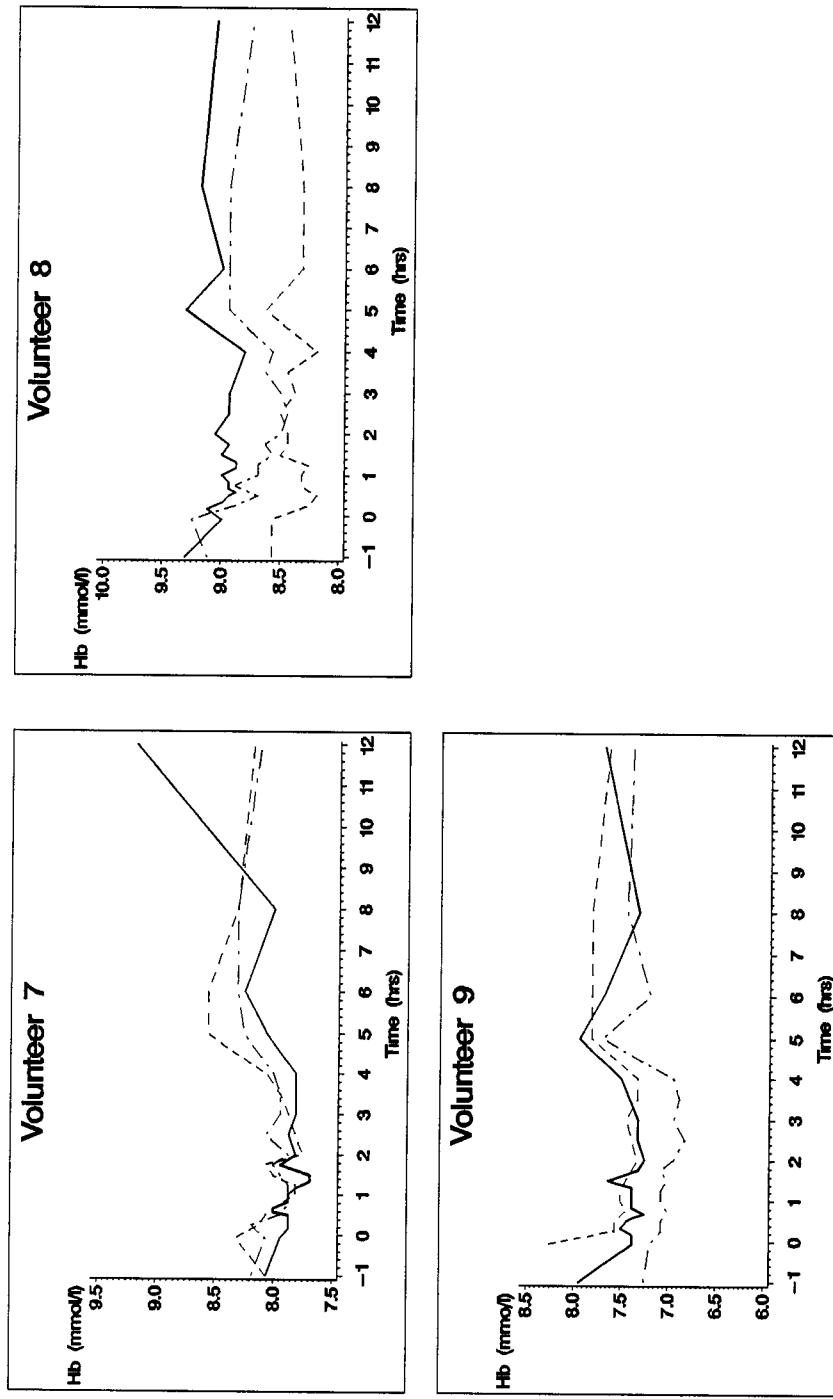


Appendix I3b Hemoglobin concentration as observed after single dose administration of sodium nitrite to adult volunteers
(continued)

(—) = Treatment A (intravenous administration of 0.12 mmol sodium nitrite per mmol Hb)

(---) = Treatment B (oral administration of 0.12 mmol sodium nitrite per mmol Hb)

(- - ·) = Treatment C (oral administration of 0.06 mmol sodium nitrite per mmol Hb)



Appendix 14a

Table of Methemoglobin Data
Treatment A = Intravenous administration of 0.12 mmol NaNO₂ per mmol Hb to adult volunteers

Volunteer number		1 TN	2 MP	3 IS	4 DL	5 JB	6 MA	7 SG	8 RV	9 EL								
Scheduled Time (hr:min)	Scheme Time (hr:min)	Actual MethHb (%)																
8.00	-1.00	-2.25	0.5	-1.58	0.4	-1.65	0.6	-2.00	0.3	-1.48	0.6	-1.17	0.5	-1.50	0.3	-1.22	0.7	
8.55	-0.08	-1.52	0.4	-0.17	0.4	-0.33	0.5	-0.42	0.3	-0.63	0.3	-0.12	0.6	-0.58	0.4	-0.20	0.7	
9.10	0.17	0.22	1.8	0.17	2.1	0.17	1.7	0.17	2.1	0.20	2.3	0.18	2.1	0.17	1.6	0.17	1.7	
9.20	0.33	0.30	5.0	0.33	4.7	0.25	4.3	0.33	4.7	0.33	4.4	0.33	4.4	0.33	3.9	0.33	3.9	
9.30	0.50	0.53	7.8	0.52	7.5	0.50	6.5	0.52	6.3	0.53	7.3	0.53	7.5	0.50	6.3	0.50	7.0	
9.35	0.58	0.63	9.0	0.60	8.2	0.62	7.6	0.60	10.0	0.60	8.5	0.73	8.8	0.60	9.2	0.58	7.8	
9.40	0.67	0.78	10.2	0.67	8.7	0.72	8.6	0.66	11.1	0.68	9.1	0.83	9.2	0.67	10.1	0.67	8.7	
9.50	0.83	0.88	11.0	0.83	9.1	0.87	9.3	0.83	12.0	1.00	10.5	1.00	10.1	0.83	10.7	0.83	9.4	
10.00	1.00	1.05	11.5	0.97	9.7	1.00	9.6	1.02	12.7	1.00	10.7	1.17	10.1	1.00	11.3	1.00	9.8	
10.10	1.17	1.22	11.8	1.17	9.4	1.20	9.4	1.17	12.6	1.17	10.8	1.33	10.2	1.18	11.4	1.17	9.9	
10.20	1.33	1.38	11.8	1.35	9.3	1.33	9.3	1.35	12.3	1.33	10.9	1.53	10.9	1.33	11.3	1.37	9.7	
10.30	1.50	1.58	11.4	1.50	9.0	1.52	8.8	1.50	11.8	1.50	10.7	1.70	10.3	1.50	11.1	1.50	9.2	
10.45	1.75	1.88	10.4	1.75	8.3	1.75	8.5	1.75	11.5	1.75	10.3	1.92	8.9	1.75	10.5	1.75	8.5	
11.00	2.00	2.05	9.9	2.00	7.6	2.02	7.6	2.02	10.4	2.02	9.7	2.17	8.2	2.00	9.8	2.00	7.6	
11.30	2.50	2.55	6.2	2.50	5.7	2.50	6.2	2.53	6.5	2.50	8.0	2.50	7.1	2.50	8.3	2.50	6.0	
12.00	3.00	3.00	6.3	3.00	4.6	3.00	4.8	2.93	7.0	3.00	6.5	3.00	5.5	3.00	6.7	3.00	4.3	
13.00	4.00	3.5	4.00	2.4	4.00	2.8	4.00	3.0	4.00	4.4	4.00	3.7	4.00	4.1	4.00	2.8	3.0	
14.00	5.00	5.00	5.00	4.98	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	
15.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	
17.00	8.00	8.00	8.00	8.00	8.00	8.05	8.05	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	
21.00	12.00	11.96	12.00	12.13	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	
21.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	
24.00	24.00	23.92	24.00	23.83	24.00	23.83	23.83	23.83	23.83	23.83	23.83	23.83	23.83	23.83	23.83	23.83	23.83	
Volunteer number		1 TN	2 MP	3 IS	4 DL	5 JB	6 MA	7 SG	8 RV	9 EL								
Scheduled Time (hr:min)	Scheme Time (hr:min)	Actual MethHb (mmol/l)																
8.00	-1.00	-2.25	0.041	-1.58	0.036	-1.50	0.048	-1.85	0.045	-2.00	0.023	-0.42	-1.48	0.044	-1.50	0.039	-1.22	0.052
8.55	-0.08	-1.52	0.033	-0.17	0.038	-0.33	0.040	-0.17	0.038	-0.42	0.023	-0.63	-0.58	0.047	-0.58	0.036	-0.20	0.052
9.10	0.17	0.22	0.148	0.17	0.189	0.17	0.135	0.17	0.144	0.17	0.160	0.20	0.168	0.17	0.164	0.17	0.125	
9.20	0.33	0.30	0.412	0.33	0.423	0.25	0.341	0.33	0.356	0.33	0.287	0.37	0.322	0.33	0.344	0.33	0.288	
9.30	0.50	0.53	0.643	0.52	0.675	0.50	0.515	0.52	0.627	0.53	0.556	0.53	0.549	0.50	0.601	0.50	0.517	
9.35	0.58	0.63	0.742	0.60	0.738	0.62	0.619	0.60	0.647	0.73	0.644	0.644	0.719	0.58	0.598	0.58	0.576	
9.40	0.67	0.76	0.840	0.67	0.753	0.72	0.682	0.68	0.839	0.68	0.850	0.63	0.673	0.67	0.769	0.67	0.642	
9.50	0.83	0.88	0.908	0.83	0.819	0.87	0.737	0.83	0.907	0.83	0.790	1.00	0.739	0.83	0.836	0.83	0.834	
10.00	1.00	1.05	0.946	1.00	0.873	1.00	0.761	1.02	0.963	1.00	0.814	1.07	0.750	1.00	0.883	1.00	0.723	
10.10	1.17	1.22	0.972	1.17	0.846	1.20	0.745	1.17	0.953	1.17	0.822	1.33	0.747	1.17	0.890	1.17	0.731	
10.20	1.33	1.38	1.020	1.33	0.956	1.35	0.837	1.35	0.930	1.33	0.820	1.53	0.725	1.33	0.883	1.33	0.716	
10.30	1.50	1.56	1.040	1.50	0.939	1.52	0.840	1.52	0.698	1.50	0.814	1.70	0.681	1.50	0.867	1.50	0.732	
10.45	1.75	1.68	0.857	1.75	0.747	1.75	0.674	1.75	0.669	1.75	0.784	1.92	0.651	1.75	0.820	1.75	0.627	
11.00	2.00	2.05	0.816	2.00	0.684	2.00	0.603	2.02	0.788	2.02	0.600	2.17	0.736	2.00	0.765	2.00	0.651	
11.30	2.55	2.55	0.676	2.50	0.513	2.50	0.492	2.53	0.643	2.50	0.669	2.50	0.520	2.50	0.648	2.50	0.536	
12.00	3.00	3.00	0.519	3.00	0.414	3.00	0.381	2.93	0.299	3.00	0.405	3.02	0.271	3.02	0.523	3.02	0.438	
13.00	4.02	4.02	0.288	4.00	0.218	4.00	0.222	4.00	0.267	4.00	0.325	4.00	0.271	4.00	0.320	4.00	0.250	
14.00	5.00	5.00	0.148	4.98	0.144	5.00	0.143	5.00	0.161	5.00	0.213	5.00	0.139	5.00	0.242	5.00	0.161	
15.00	6.00	6.00	0.066	6.00	0.081	6.00	0.119	6.00	0.121	6.00	0.120	5.92	0.117	6.00	0.159	6.00	0.107	
17.00	8.00	8.00	0.041	8.00	0.090	8.05	0.056	8.00	0.053	8.00	0.081	8.10	0.037	8.03	0.070	8.03	0.080	
21.00	12.00	11.98	0.033	12.13	0.054	12.00	0.045	12.00	0.045	12.00	0.061	12.00	0.029	12.00	0.036	12.00	0.037	
1.00	16.00	16.00	0.016	16.00	0.018	15.95	0.048	16.00	0.030	16.00	0.030	16.17	0.044	16.00	0.045	16.00	0.039	
9.00	24.00	23.92	0.033	23.83	0.027	23.95	0.024	23.55	0.045	23.45	0.015	24.00	0.016	24.00	0.015	24.00	0.015	

**Appendix 14a Table of Methemoglobin Data
(continued)**

Treatment B = Oral administration of 0.12 mmol NaNO₂ per mmol Hb to adult volunteers

Volunteer number	1 TN	2 MP	3 IS	4 DL	5 JB	6 MR	7 SG	8 RV	9 EL
Scheduled Time (hr:min)	Scheme Time (hr:min)	MethHb Actual Time (hr:min) (%)							
8.00	-1.00	-1.25	0.4	-1.55	0.4	-0.83	0.2	-1.08	0.5
8.55	-0.08	-0.03	0.5	-0.55	0.3	-0.08	0.5	-0.77	0.3
9.15	0.25	0.25	2.4	0.25	3.8	0.25	1.2	0.25	4.5
9.30	0.50	0.50	6.6	0.50	7.5	0.50	2.8	0.48	6.7
9.45	0.75	0.75	9.7	0.75	8.9	0.75	3.9	0.80	8.7
10.00	1.00	1.00	10.7	1.00	9.2	1.00	5.9	1.00	9.5
10.15	1.25	1.25	11.3	1.25	9.1	1.25	7.7	1.25	10.9
10.30	1.50	1.50	11.1	1.50	8.2	1.50	8.0	1.50	9.4
10.45	1.75	1.75	10.5	1.80	7.4	1.75	7.9	1.75	8.7
11.00	2.00	2.00	9.8	2.03	7.0	2.00	7.6	2.00	8.1
11.30	2.50	2.50	7.8	2.52	7.5	2.50	6.2	2.50	6.6
12.00	3.00	3.00	6.3	3.00	4.1	3.00	5.0	3.00	4.8
12.30	3.50	3.50	4.5	3.52	2.9	3.50	3.7	3.52	3.7
13.00	4.00	4.00	4.00	4.03	2.2	4.00	2.9	4.00	2.7
14.00	5.00	5.00	5.00	5.02	1.8	5.00	1.9	5.00	1.8
15.00	6.00	6.02	6.02	6.02	1.1	6.00	0.9	5.98	1.1
17.00	8.00	8.00	8.00	8.13	0.6	8.00	0.5	8.12	1.1
21.00	12.00	12.00	11.98	11.98	0.6	12.00	0.6	12.10	0.5
1.00	16.00	16.00	16.00	16.00	0.1	15.98	0.2	16.08	0.1
9.00	24.00	24.00	23.93	23.93	0.1	23.83	0.4	23.75	0.7

Volunteer number	1 TN	2 MP	3 IS	4 DL	5 JB	6 MR	7 SG	8 RV	9 EL
Scheduled Time (hr:min)	Scheme Time (hr:min)	MethHb Actual Time (mmol/l) (hr:min)							
8.00	-1.00	-1.25	0.032	-1.55	0.037	-0.83	0.016	-1.08	0.040
8.55	-0.08	-0.03	0.040	-0.55	0.027	-0.08	0.041	-0.77	0.022
9.15	0.25	0.25	0.347	0.25	0.25	0.099	0.25	0.262	0.25
9.30	0.50	0.50	0.527	0.50	0.686	0.50	0.230	0.48	0.501
9.45	0.75	0.75	0.774	0.75	0.813	0.75	0.320	0.80	0.651
10.00	1.00	1.00	0.854	1.00	0.841	1.00	0.484	1.00	0.711
10.15	1.25	1.25	0.902	1.25	0.832	1.25	0.632	1.25	0.733
10.30	1.50	1.50	0.886	1.50	0.749	1.50	0.657	1.50	0.703
10.45	1.75	1.75	0.836	1.80	0.676	1.75	0.649	1.75	0.651
11.00	2.00	2.00	0.782	2.03	0.640	2.00	0.624	2.00	0.606
11.30	2.50	2.50	0.622	2.52	0.503	2.50	0.509	2.50	0.494
12.00	3.00	3.00	0.503	3.00	0.375	3.00	0.411	3.00	0.462
12.30	3.50	3.50	0.359	3.52	0.265	3.50	0.304	3.52	0.277
13.00	4.00	4.00	0.295	4.03	0.201	4.00	0.238	4.00	0.202
14.00	5.00	5.00	0.144	5.02	0.119	5.00	0.156	5.00	0.097
15.00	6.00	6.02	0.064	6.02	0.101	6.00	0.067	5.98	0.120
17.00	8.00	8.00	0.072	8.13	0.055	8.00	0.041	8.12	0.086
21.00	12.00	12.00	0.056	11.98	0.055	12.00	0.049	12.10	0.060
1.00	16.00	16.00	0.056	16.00	0.009	15.98	0.018	16.08	0.040
9.00	24.00	24.00	0.008	23.93	0.018	23.83	0.033	23.75	0.052

ND = not determined

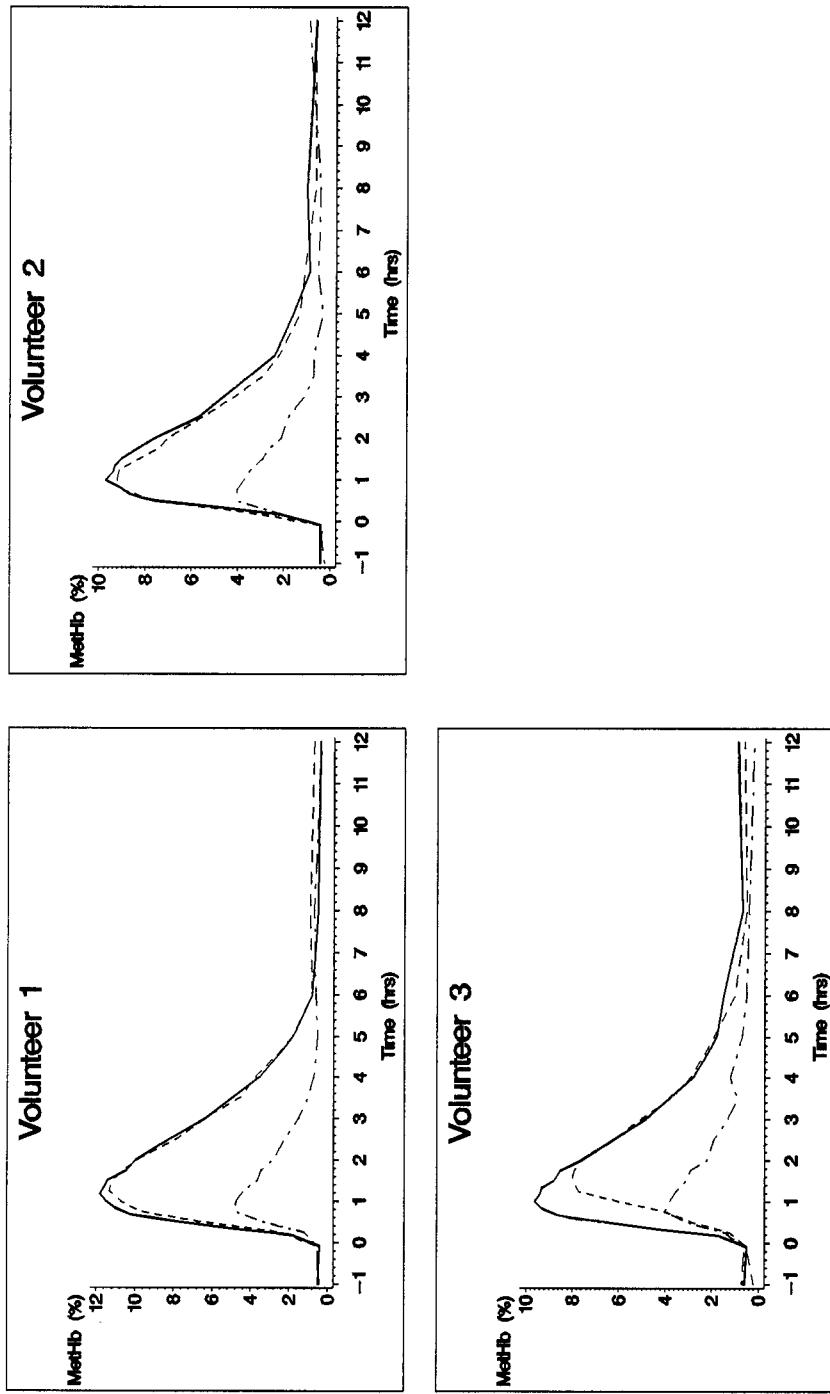
**Appendix 14a Table of Methemoglobin Data
(continued)**

Treatment C = Oral administration of 0.06 mmol NaNO₂ per mmol Hb to adult volunteers

Volunteer number		1 TN		2 MP		3 IS		4 DL		5 JB		6 MR		7 SG		8 RV		9 EL	
Scheduled Time (hr:min)	Scheme Time (hr:min)	Actual Time (hr:min)	MethHb (%)																
8.00	-1.00	-1.55	0.5	-1.58	0.2	-3.00	0.7	-1.87	0.5	-1.25	0.6	-0.83	0.4	-1.67	0.5	-1.58	0.2	-1.08	0.2
8.55	-0.08	-0.08	0.5	-0.12	0.4	-1.38	0.6	-0.72	0.6	-0.42	0.7	-0.75	0.2	-0.08	0.4	-0.08	0.4	-0.08	0.3
9.15	0.25	1.2	0.17	2.5	0.25	1.4	0.25	1.5	0.25	1.4	0.25	1.4	0.30	3.3	0.25	3.1	0.25	3.3	
9.30	0.50	3.3	0.53	4.0	0.50	3.0	0.50	3.4	0.50	4.6	0.50	2.6	0.50	4.5	0.50	4.1	0.50	4.7	
9.45	0.75	0.75	4.8	0.75	4.0	0.75	4.0	0.77	4.3	0.77	4.7	0.75	3.3	0.75	4.8	0.75	3.9	0.75	
10.00	1.00	1.00	4.7	1.00	3.7	1.00	4.0	1.00	4.0	1.00	4.4	1.00	3.9	1.00	4.5	1.00	3.8	1.00	
10.15	1.25	1.25	4.3	1.25	3.4	1.25	3.5	1.27	3.6	1.25	4.3	1.25	3.9	1.25	4.1	1.25	3.4	1.25	
10.30	1.50	1.52	3.6	1.50	2.9	1.50	3.1	1.50	3.3	1.50	3.9	1.50	3.2	1.50	3.1	1.50	3.1	1.50	
10.45	1.75	1.78	3.4	1.73	2.6	1.77	2.9	1.77	3.0	1.75	3.3	1.75	2.7	1.75	3.1	1.75	2.8	1.77	
11.00	2.00	2.00	2.8	1.98	2.1	2.02	2.2	2.00	2.6	2.00	3.0	1.98	2.8	2.00	2.7	2.00	2.5	2.00	
11.30	2.50	2.50	2.2	2.50	1.8	2.50	1.9	2.52	1.5	2.25	2.7	2.50	2.2	2.50	1.7	2.50	1.8	2.50	
12.00	3.00	3.00	1.5	3.00	1.1	3.00	1.3	3.00	1.4	2.92	1.6	3.00	1.3	3.03	1.4	3.00	1.3	3.00	
12.30	3.50	3.50	1.0	3.50	0.7	3.50	0.9	3.50	1.0	3.50	1.0	3.48	1.0	3.50	1.1	3.50	1.2	3.50	
13.00	4.00	4.00	0.7	4.00	1.2	4.00	1.2	4.00	1.1	4.00	1.1	4.00	1.0	4.00	1.0	4.00	0.8	4.00	
14.00	5.00	5.00	0.5	5.00	0.3	5.00	0.7	5.00	0.8	5.00	0.7	5.00	0.7	5.00	0.7	5.00	0.6	5.00	
15.00	6.00	6.00	0.6	6.00	0.5	6.00	0.5	6.00	0.6	6.02	0.6	6.00	0.6	6.03	0.5	6.00	0.4	6.05	
17.00	8.00	8.00	0.7	8.00	0.4	8.08	0.4	8.12	0.3	8.00	0.6	8.00	0.6	8.02	0.5	8.00	0.7	7.95	
21.00	12.00	12.17	0.3	12.00	0.9	12.00	0.2	12.05	0.5	12.03	0.7	11.97	0.6	12.00	0.4	12.00	0.8	12.00	
1.00	16.00	16.03	0.3	15.97	0.4	16.00	0.6	15.92	0.5	16.08	0.6	16.00	0.4	16.08	0.4	15.98	0.4	15.98	
9.00	24.00	23.83	0.2	23.92	0.4	24.00	0.3	23.25	0.3	23.57	0.1	23.75	0.4	24.08	0.3	23.95	0.6	23.95	
Volunteer number		1 TN		2 MP		3 IS		4 DL		5 JB		6 MR		7 SG		8 RV		9 EL	
Scheduled Time (hr:min)	Scheme Time (hr:min)	Actual Time (hr:min)	MethHb (mmol/l)																
8.00	-1.00	-1.55	0.039	-1.58	0.018	-3.00	0.055	-1.87	0.040	-1.25	0.046	-0.83	0.030	-1.67	0.040	-1.58	0.017	-1.08	0.014
8.55	-0.08	-0.08	0.039	-0.12	0.035	-1.38	0.047	-0.72	0.048	-0.42	0.054	-0.25	0.015	-0.75	0.040	-0.08	0.034	-0.08	0.021
9.15	0.25	0.25	0.094	0.17	0.219	0.25	0.110	0.25	0.119	0.25	0.25	0.106	0.30	0.262	0.25	0.26	0.25	0.230	
9.30	0.50	0.50	0.258	0.53	0.351	0.50	0.237	0.50	0.270	0.50	0.356	0.50	0.197	0.50	0.357	0.50	0.351	0.50	0.328
9.45	0.75	0.75	0.376	0.75	0.351	0.75	0.316	0.77	0.341	0.77	0.364	0.75	0.249	0.75	0.381	0.75	0.334	0.75	0.321
10.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
10.15	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.27	1.28	1.27	1.28	1.25	1.30	1.295	1.25	1.326	1.00	1.295	1.25
10.30	1.50	1.52	1.52	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
10.45	1.75	1.78	1.78	1.73	1.73	1.77	1.77	1.77	1.77	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75
11.00	2.00	2.00	2.00	2.19	1.98	2.02	2.02	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
11.30	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.52	2.52	2.52	2.52	2.52	2.52	2.52	2.52	2.52	2.52	2.52	2.52
12.00	3.00	3.00	0.117	3.00	0.097	3.00	0.103	3.00	0.111	2.92	0.124	3.00	0.098	3.03	0.111	3.00	0.105	3.00	0.105
12.30	3.50	3.50	0.078	3.50	0.061	3.50	0.071	3.50	0.071	3.50	0.077	3.48	0.076	3.50	0.087	3.50	0.103	3.50	0.091
13.00	4.00	4.00	0.055	4.00	0.061	4.00	0.095	4.00	0.071	4.00	0.085	4.00	0.060	4.00	0.079	4.00	0.089	4.00	0.086
14.00	5.00	5.00	0.039	5.00	0.026	5.00	0.055	5.00	0.064	5.00	0.054	4.98	0.038	5.00	0.056	5.00	0.056	5.00	0.056
15.00	6.00	6.00	0.047	6.00	0.044	6.00	0.039	6.00	0.048	6.02	0.048	6.00	0.053	6.03	0.040	6.00	0.054	6.05	0.021
16.00	8.00	8.00	0.095	8.00	0.095	8.00	0.032	8.12	0.024	8.00	0.046	8.00	0.045	8.02	0.040	8.00	0.056	7.95	0.035
17.00	12.00	12.17	0.023	12.00	0.016	12.00	0.016	12.05	0.040	12.03	0.034	11.97	0.045	11.97	0.048	12.00	0.034	12.00	0.056
1.00	16.00	16.03	0.023	15.97	0.035	16.00	0.047	15.92	0.040	16.08	0.039	16.12	0.045	16.00	0.032	16.08	0.039	15.98	0.028
9.00	24.00	23.83	0.016	23.92	0.035	24.00	0.024	23.25	0.024	23.57	0.008	23.75	0.008	24.08	0.024	24.03	0.034	23.95	0.042

Appendix 14b *Methemoglobin concentration as observed after single dose administration of sodium nitrite to adult volunteers*

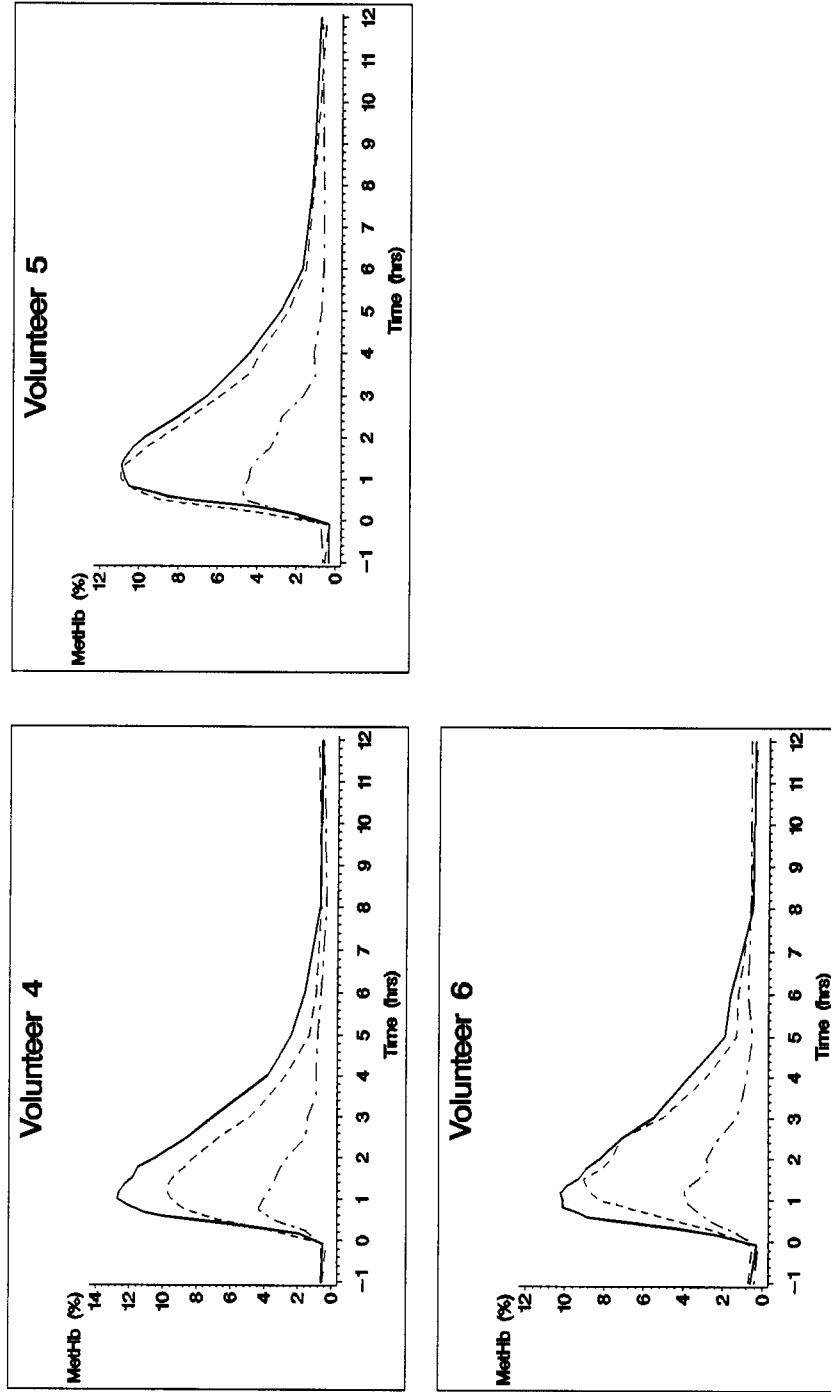
- (—) = Treatment A (intravenous administration of 0.12 mmol sodium nitrite per mmol Hb)
(---) = Treatment B (oral administration of 0.12 mmol sodium nitrite per mmol Hb)
(-·-) = Treatment C (oral administration of 0.06 mmol sodium nitrite per mmol Hb)



Appendix 14b
(continued)

Methemoglobin concentration as observed after single dose administration of sodium nitrite to adult volunteers

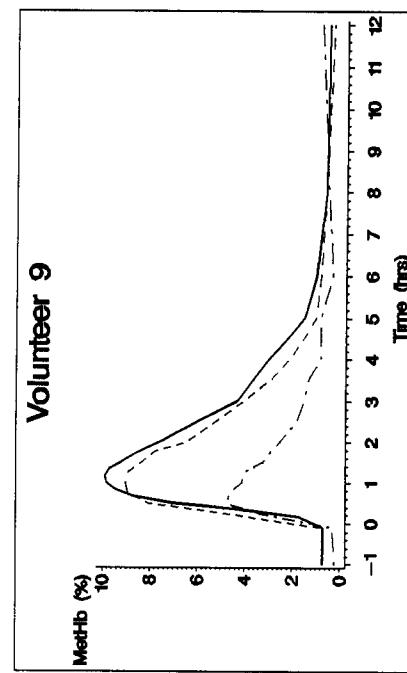
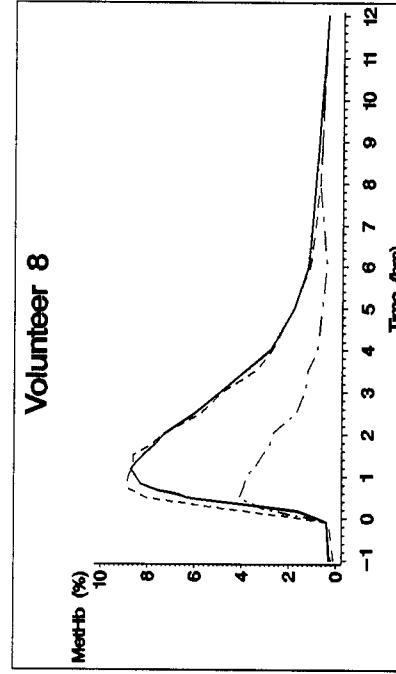
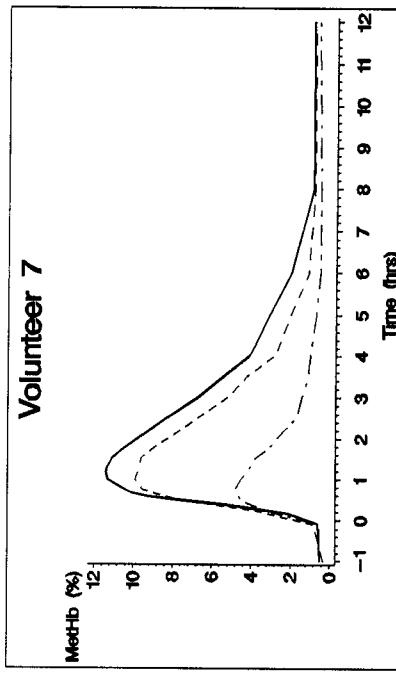
- (—) = Treatment A (intravenous administration of 0.12 mmol sodium nitrite per mmol Hb)
- (---) = Treatment B (oral administration of 0.12 mmol sodium nitrite per mmol Hb)
- (—·—) = Treatment C (oral administration of 0.06 mmol sodium nitrite per mmol Hb)



Appendix 14b
(continued)

Methemoglobin concentration as observed after single dose administration of sodium nitrite to adult volunteers

- (—) = Treatment A (intravenous administration of 0.12 mmol sodium nitrite per mmol Hb)
- (---) = Treatment B (oral administration of 0.12 mmol sodium nitrite per mmol Hb)
- (-·-) = Treatment C (oral administration of 0.06 mmol sodium nitrite per mmol Hb)



Appendix 15 Table of kinetic parameters derived from the Methemoglobin Data

Treatment A						
Volunteer number	Nitrite dose (mg)	C-max (mmol/L)	C-max adjusted*	t-max (hrs)	t-1/2 (hrs)	Corr coeff (t-1/2)
1 TN	350	0.935	0.855	1.22	0.98	1.00
2 MP	380	0.841	0.708	1.00	1.14	0.99
3 IS	290	0.720	0.754	1.00	1.16	1.00
4 DL	350	0.922	0.894	1.02	1.20	1.00
5 JB	310	0.794	0.820	1.33	1.36	0.99
6 MR	300	0.714	0.762	1.33	1.28	0.98
7 SG	300	0.848	0.905	1.18	1.48	0.99
8 RV	360	0.752	0.658	1.17	1.28	1.00
9 EL	290	0.691	0.762	1.17	1.05	0.98
Mean	323	0.802	0.796	1.16	1.21	1.00
					2.497	3.3

Treatment B						
Volunteer number	Nitrite dose (mg)	C-max (mmol/L)	C-max adjusted*	t-max (hrs)	t-1/2 (hrs)	Corr coeff (t-1/2)
1 TN	350	0.865	0.791	1.25	1.02	0.98
2 MP	380	0.809	0.881	1.00	1.01	1.00
3 IS	315	0.650	0.650	1.50	1.21	1.881
4 DL	330	0.695	0.674	1.25	1.04	1.00
5 JB	310	0.834	0.861	1.00	1.31	1.2
6 MR	300	0.637	0.679	1.20	1.00	1.994
7 SG	300	0.740	0.789	1.02	1.18	0.98
8 RV	360	0.721	0.641	0.75	2.279	9
9 EL	290	0.626	0.691	0.98	0.99	0.99
Mean	323	0.727	0.721	1.14	1.13	0.99
				2.169	2.152	0.87
						(0.86)

Treatment C						
Volunteer number	Nitrite dose (mg)	C-max (mmol/L)	C-max adjusted*	t-max (hrs)	t-1/2 (hrs)	correlate coefficient
1 TN	170	0.339	0.638	0.75	0.91	0.98
2 MP	190	0.319	0.537	0.53	1.19	0.95
3 IS	140	0.275	0.629	0.75	1.04	0.93
4 DL	160	0.303	0.606	0.77	1.21	0.97
5 JB	160	0.329	0.658	0.77	1.24	0.98
6 MR	150	0.262	0.559	1.00	1.21	1.00
7 SG	150	0.339	0.723	0.75	0.84	0.98
8 RV	180	0.326	0.580	0.50	1.03	0.92
9 EL	150	0.288	0.614	0.50	0.95	0.99
Mean	161	0.309		0.70	1.07	0.690
						1.372
						0.56

Treatment B						
Parameter	Treatment A versus Treatment B			Treatment A versus Treatment C		
AUC	0.0084 (0.0058)			0.0001 *		
t-1/2	0.085			0.065		
t-max	0.85			0.0001		
C-max	0.015 (0.0049)			0.0001 *		

* = corrected for difference in the administered sodium nitrite dose
 () = results of volunteer 5 excluded

adjusted* = results adjusted to a standard dose of 320 mg sodium nitrite

Appendix 16a Table of Plasma Nitrite Concentrations

Treatment A = Intravenous administration of 0.12 mmol NaNO₂ per mmol Hb to adult volunteers

Volunteer number	1 TN	2 MP	3 IS	4 DL	5 JB	6 MR	7 SG	8 RV	9 EL
Scheduled Time (hr:min)	Scheme Time (hr)	Nitrite Actual Time (hr)							
	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
8.00	-2.25	<0.1	-1.58	<0.1	-1.85	<0.1	-2.00	<0.1	-1.48
8.55	-0.08	<0.1	-0.17	<0.1	-0.33	0.1	-0.17	<0.1	-0.42
9.10	0.17	0.22	0.6	0.17	2.6	1.3	1.3	0.20	0.17
9.20	0.33	0.30	** 3.4	0.33	3.1	0.25	1.9	0.33	1.7
9.30	0.50	0.53	2.8	0.52	3.7	0.50	1.9	0.53	2.7
9.35	0.58	0.63	2.6	0.60	2.8	0.62	2.3	0.60	2.0
9.40	0.67	0.78	2.4	0.67	1.8	0.72	1.7	0.68	1.3
9.50	0.83	0.88	1.6	0.83	1.4	0.87	1.4	0.83	1.0
10.00	1.00	1.05	1.5	1.00	1.0	1.00	1.1	1.02	1.0
10.10	1.17	1.22	0.8	1.17	0.8	1.20	0.8	1.17	0.8
10.20	1.33	1.38	0.8	1.35	0.5	1.33	0.5	1.35	0.7
10.30	1.50	1.58	0.6	1.50	0.4	1.52	0.4	1.50	0.6
10.45	1.75	1.88	0.4	1.75	0.3	1.75	0.3	1.75	0.4
11.00	2.00	2.05	0.3	2.00	0.2	2.00	0.3	2.02	0.3
11.30	2.50	2.55	0.2	2.50	<0.1	2.50	0.2	2.53	0.2
12.00	3.00	3.00	0.1	3.00	<0.1	2.93	0.1	3.00	0.1
13.00	4.00	4.02	<0.1	4.00	<0.1	4.00	<0.1	4.00	<0.1
14.00	5.00	5.00	<0.1	4.98	<0.1	5.00	<0.1	5.00	<0.1
15.00	6.00	6.05	<0.1	6.00	<0.1	6.00	<0.1	5.92	<0.1
17.00	8.00	8.00	<0.1	8.00	<0.1	8.05	<0.1	8.10	<0.1
21.00	12.00	11.98	-	12.13	<0.1	12.00	<0.1	12.03	<0.1
1.00	16.00	16.00	<0.1	16.00	* <0.1	15.92	<0.1	16.08	<0.1
9.00	24.00	23.92	<0.1	23.83	<0.1	23.95	<0.1	23.75	<0.1

* = mild hemolysis; ** = moderate hemolysis; *** = major hemolysis

- = missing sample

Appendix 16a **Table of Plasma Nitrite Concentrations**
 (continued) Treatment B = Oral administration of 0.12 mmol NaNO₂ per mmol Hb to adult volunteers

Volunteer number	1 TN			2 MP			3 IS			4 DL			5 JB			6 MR			7 SG			8 RV		
	Scheduled Time (hr:min)	Scheme Time (hr)	Nitrite (mg/kg)	Actual Time (hr)	Nitrite (mg/kg)																			
8.00	-1.00	-1.25	<0.1	-1.55	<0.1	-0.83	<0.1	-1.08	<0.1	-1.78	<0.1	-0.92	<0.1	-1.17	<0.1	-1	<0.1	-1.17	<0.1	-1.00	<0.1	-1.00	<0.1	
8.55	-0.08	-0.03	<0.1	-0.55	<0.1	-0.08	0.1	-0.77	<0.1	-0.67	<0.1	-0.38	<0.1	-0.08	<0.1	-0.17	<0.1	-0.42	<0.1	-0.42	<0.1	-0.42	<0.1	
9.15	0.25	0.25	1.8	0.25	0.25	2.7	0.5	0.25	0.25	2.4	0.25	1.2	0.25	3.0	0.25	3.6	0.25	3.3	0.25	3.3	0.25	3.3		
9.30	0.50	0.50	3.1	0.50	0.50	2.0	0.50	0.50	0.9	0.48	2.5	0.47	2.3	0.50	1.9	0.50	2.4	0.50	1.5	0.50	1.5	0.50	2.4	
9.45	0.75	0.75	2.5	0.75	0.75	1.8	0.75	1.3	0.80	1.9	0.75	1.4	0.75	1.9	0.78	1.9	0.75	0.9	0.75	0.9	0.75	0.9	1.6	
10.00	1.00	1.00	1.9	1.00	1.00	0.8	1.00	1.2	1.00	1.00	1.3	1.00	1.1	1.00	1.5	1.02	1.3	1.00	0.6	0.98	1.2	0.98	1.2	
10.15	1.25	1.25	1.1	1.25	1.25	0.5	1.25	1.2	1.25	0.9	1.25	0.9	1.25	1.3	1.30	0.8	1.25	0.4	1.25	0.7	1.25	0.7	1.25	
10.30	1.50	1.50	0.8	1.50	1.50	0.4	1.50	1.0	1.50	0.8	1.50	0.6	1.50	1.0	1.53	0.4	1.50	0.2	1.50	0.4	1.50	0.4	1.50	
10.45	1.75	1.75	0.7	1.80	1.80	0.3	1.75	0.6	1.75	0.3	1.75	0.4	1.75	0.5	1.60	0.4	1.75	0.2	1.75	0.2	1.75	0.2	1.75	
11.00	2.00	2.00	0.5	2.03	2.00	0.2	2.00	0.3	2.00	0.3	2.00	0.3	2.00	0.3	2.00	0.2	2.00	0.1	2.00	0.2	2.00	0.2	2.00	
11.30	2.50	2.50	0.3	2.52	0.1	2.50	0.2	2.50	0.1	2.50	0.1	2.23	0.4	2.50	0.1	2.50	<0.1	2.50	<0.1	2.50	<0.1	2.50	<0.1	
12.00	3.00	3.00	0.2	3.00	<0.1	3.00	0.2	3.00	<0.1	3.00	0.1	2.92	0.1	3.00	<0.1	3.00	<0.1	3.00	<0.1	3.00	<0.1	3.00	<0.1	
12.30	3.50	3.50	0.1	3.52	<0.1	3.50	0.1	3.52	<0.1	3.50	<0.1	3.50	<0.1	3.50	<0.1	3.50	<0.1	3.50	<0.1	3.50	<0.1	3.50	<0.1	
13.00	4.00	4.00	0.1	4.03	4.00	<0.1	4.00	<0.1	4.00	<0.1	4.00	<0.1	3.98	<0.1	4.00	<0.1	3.98	<0.1	3.98	<0.1	3.98	<0.1	3.98	<0.1
14.00	5.00	5.00	0.1	5.02	<0.1	5.00	<0.1	5.00	<0.1	5.00	<0.1	4.97	<0.1	5.00	<0.1	5.02	<0.1	4.98	<0.1	4.98	<0.1	4.98	<0.1	
15.00	6.00	6.02	<0.1	6.02	<0.1	6.00	<0.1	6.00	<0.1	5.98	<0.1	6.00	<0.1	6.03	<0.1	5.98	<0.1	6.03	<0.1	5.98	<0.1	6.03	<0.1	
17.00	8.00	8.00	<0.1	8.13	** 0.2	8.00	<0.1	8.00	<0.1	8.12	<0.1	8.08	<0.1	8.00	<0.1	7.95	<0.1	7.95	<0.1	7.95	<0.1	7.95	<0.1	
21.00	12.00	12.00	<0.1	11.98	<0.1	12.00	<0.1	12.00	<0.1	12.10	<0.1	12.08	<0.1	12.00	<0.1	11.98	<0.1	11.98	<0.1	12.00	<0.1	12.00	<0.1	
1.00	16.00	16.00	<0.1	16.00	<0.1	16.06	<0.1	16.06	<0.1	15.92	<0.1	16.02	<0.1	16.00	<0.1	16.07	*+ 2.9	16.07	*+ 2.9	16.08	*+ 2.9	16.08	*+ 2.9	
9.00	24.00	24.00	<0.1	23.93	<0.1	23.83	<0.1	23.75	<0.1	23.08	<0.1	23.55	<0.1	24.00	<0.1	24.00	<0.1	24.00	<0.1	24.00	<0.1	24.00	<0.1	

* = mild hemolyses; ** = moderate hemolyses; *** = major hemolyses

-- = missing sample

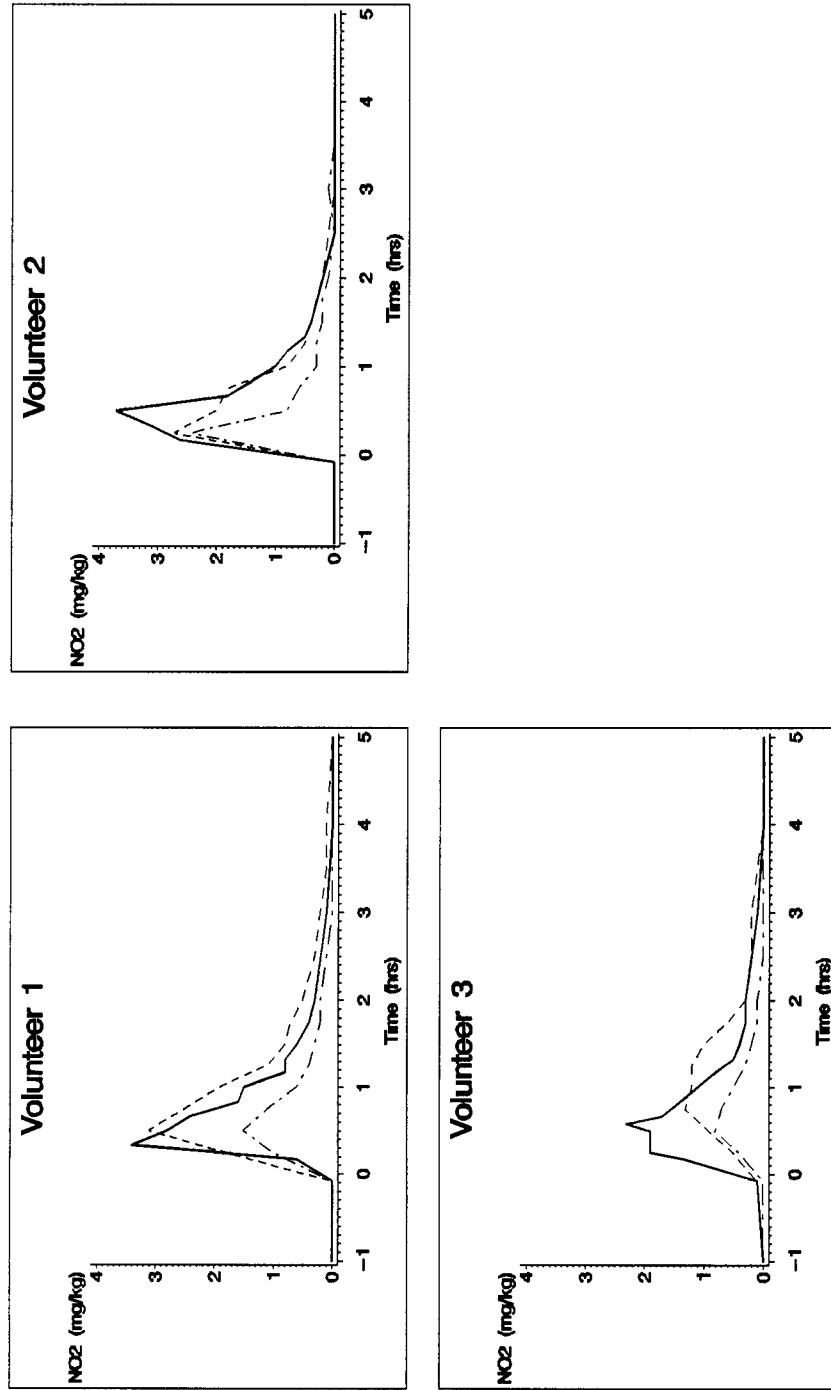
**Appendix 16a Table of Plasma Nitrite Concentrations
(continued) Treatment C = Oral administration of 0.06 mmol NaNO₂ per mmol Hb to adult volunteers**

Volunteer number	1 TN	2 MP	3 IS	4 DL	5 JB	6 MR	7 SG	8 RV	9 EL
Scheduled Time (hr:min)	Scheme Time (hr)	Actual Nitrite (mg/kg)	Actual Time (hr)	Nitrite (mg/kg)	Actual Time (hr)	Nitrite (mg/kg)	Actual Time (hr)	Nitrite (mg/kg)	Actual Time (hr)
									Nitrite (mg/kg)
8.00	-1.00	-1.55	<0.1	-1.58	<0.1	-1.87	<0.1	-1.25	<0.1
8.55	-0.08	-0.08	<0.1	-0.12	<0.1	-0.72	<0.1	-0.42	<0.1
9.15	0.25	0.25	1.0	0.17	2.4	0.25	0.8	2.3	0.25
9.30	0.50	0.50	1.5	0.53	0.8	0.50	1.0	1.4	0.50
9.45	0.75	0.75	1.1	0.75	0.6	0.75	0.7	0.77	0.9
10.00	1.00	1.00	0.6	1.00	0.3	1.00	0.5	1.00	0.6
10.15	1.25	1.25	0.4	1.25	0.3	1.27	0.4	1.25	0.4
10.30	1.50	1.52	0.3	1.50	0.2	1.50	0.2	1.50	0.3
10.45	1.75	1.78	0.2	1.73	0.2	1.77	0.1	1.77	0.2
11.00	2.00	2.00	0.2	1.98	0.1	2.02	0.1	2.00	0.2
11.30	2.50	2.50	0.1	2.50	<0.1	2.52	0.1	2.25	<0.1
12.00	3.00	3.00	0.1	3.00	<0.1	3.00	<0.1	2.92	<0.1
12.30	3.50	3.50	<0.1	3.50	<0.1	3.50	<0.1	3.50	<0.1
13.00	4.00	4.00	<0.1	4.00	<0.1	4.00	<0.1	4.00	<0.1
14.00	5.00	5.00	<0.1	5.00	<0.1	5.00	<0.1	4.98	<0.1
15.00	6.00	6.00	<0.1	6.00	<0.1	6.00	<0.1	6.02	0.1
17.00	8.00	8.00	<0.1	8.08	<0.1	8.12	<0.1	8.00	<0.1
21.00	12.00	12.00	<0.1	12.00	<0.1	12.03	<0.1	11.97	<0.1
1.00	16.00	16.03	<0.1	15.97	<0.1	16.08	<0.1	16.12	* <0.1
9.00	24.00	23.83	<0.1	23.92	<0.1	23.25	<0.1	23.75	<0.1
								24.08	<0.1
								24.03	<0.1

* = mild hemolyses; ** = moderate hemolyses; *** = major hemolyses

Appendix 16b Plasma Nitrite concentration as observed after single dose administration of sodium nitrite to adult volunteers

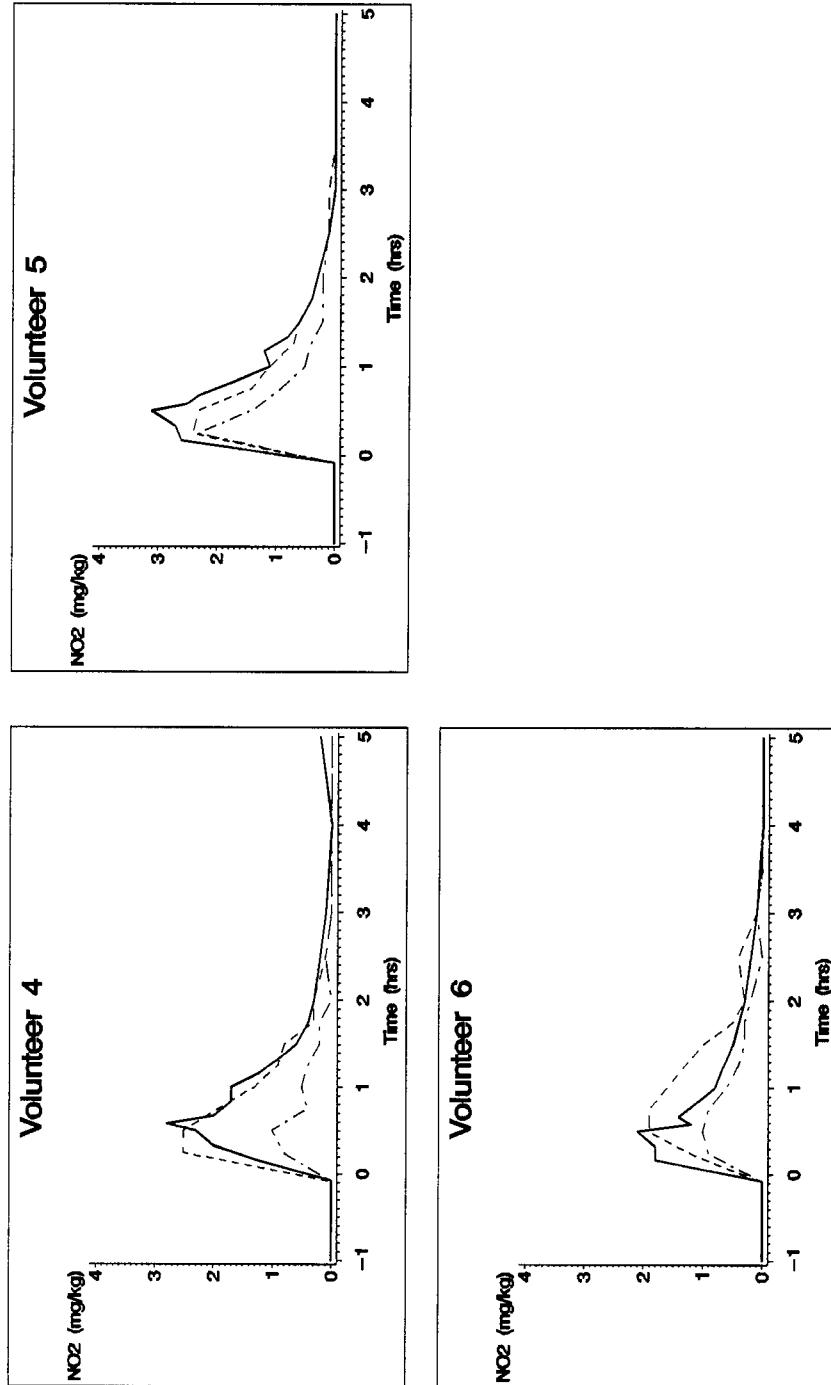
- (—) = Treatment A (intravenous administration of 0.12 mmol sodium nitrite per mmol Hb)
(---) = Treatment B (oral administration of 0.12 mmol sodium nitrite per mmol Hb)
(-·-) = Treatment C (oral administration of 0.06 mmol sodium nitrite per mmol Hb)



Appendix 16b
(continued)

Plasma Nitrite concentration as observed after single dose administration of sodium nitrite to adult volunteers

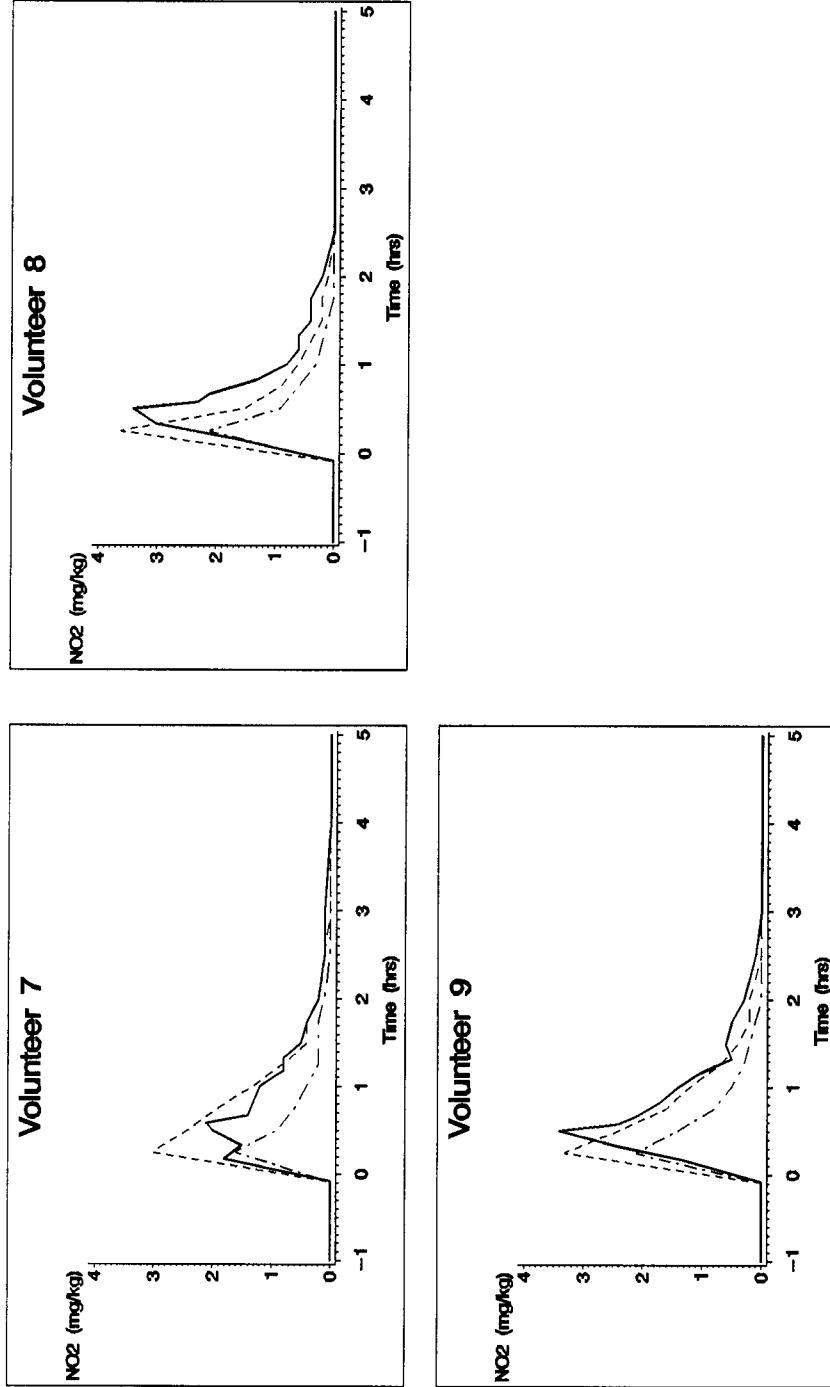
- (—) = Treatment A (intravenous administration of 0.12 mmol sodium nitrite per mmol Hb)
- (---) = Treatment B (oral administration of 0.12 mmol sodium nitrite per mmol Hb)
- (- - -) = Treatment C (oral administration of 0.06 mmol sodium nitrite per mmol Hb)



Appendix 16b
(continued)

Plasma Nitrite concentration as observed after single dose administration of sodium nitrite to adult volunteers

- (—) = Treatment A (intravenous administration of 0.12 mmol sodium nitrite per mmol Hb)
- (---) = Treatment B (oral administration of 0.12 mmol sodium nitrite per mmol Hb)
- (- · -) = Treatment C (oral administration of 0.06 mmol sodium nitrite per mmol Hb)



Appendix 17 Table of kinetic parameters derived from the Plasma Nitrite Concentrations

Treatment A									
Volunteer number	Nitrite dose (mg)	C-max (mg/kg)	C-max adjusted*	t-max (hrs)	t-1/2 (hrs)	Corr. coef. AUC(0- <i>inf</i>) (mg hr/l)	Extrapolation AUC (%) adjusted*	AUC (%) adjusted*	Vz (l)
1 TN	350	3.4	3.1	0.30	0.56	0.97	2.962	8	2.708
2 MP	380	3.7	3.1	0.52	0.42	0.96	2.811	6	2.367
3 IS	290	2.3	2.5	0.62	0.60	0.84	2.334	11	2.575
4 DL	330	2.8	2.7	0.60	0.41	0.98	2.697	7	2.615
5 JB	310	3.1	3.2	0.53	0.43	0.98	3.032	6	3.130
6 MR	300	2.1	2.2	0.53	0.71	1.00	2.466	12	1.24
7 SG	300	2.1	2.2	0.60	0.51	0.91	2.281	13	2.433
8 RV	360	3.4	3.0	0.50	0.56	0.77	2.663	12	2.367
9 EL	290	3.4	3.8	0.50	0.58	0.81	2.815	9	3.106
Mean	323	2.9	2.9	0.52	0.53	0.91	2.674	2.659	93

Treatment B									
Volunteer number	Nitrite dose (mg)	C-max (mg/kg)	C-max adjusted*	t-max (hrs)	t-1/2 (hrs)	Corr. coef. AUC(0- <i>inf</i>) (mg hr/l)	Extrapolation AUC (%) adjusted*	AUC (%) adjusted*	Bioavailability (F)
1 TN	350	3.1	2.8	0.50	0.52	0.99	1.420	16	2.673
2 MP	380	3.1	4.0	0.17	0.50	0.75	1.398	16	2.253
3 IS	140	0.8	1.8	0.50	0.34	1.00	0.784	19	1.792
4 DL	160	1.0	2.0	0.50	0.38	0.89	0.919	12	1.838
5 JB	160	2.3	4.6	0.25	0.41	0.93	1.572	15	3.144
6 MR	150	1.0	2.1	0.50	0.73	0.87	1.379	23	2.942
7 SG	150	1.6	3.4	0.30	0.32	0.98	0.969	9	2.067
8 RV	180	2.1	3.7	0.25	0.25	1.00	1.046	10	1.860
9 EL	150	2.1	4.5	0.25	0.35	1.00	1.390	11	2.965
Mean	161	1.6	3.2	0.36	0.42	0.93	1.202	2.393	0.89

Treatment C									
Volunteer number	Nitrite dose (mg)	C-max (mg/kg)	C-max adjusted*	t-max (hrs)	t-1/2 (hrs)	Corr. coef. AUC(0- <i>inf</i>) (mg hr/l)	Extrapolation AUC (%) adjusted*	AUC (%) adjusted*	Bioavailability (F)
1 TN	170	1.5	2.8	0.50	0.52	0.99	1.420	16	2.673
2 MP	190	2.4	4.0	0.17	0.50	0.75	1.398	16	2.253
3 IS	140	0.8	1.8	0.50	0.34	1.00	0.784	19	1.792
4 DL	160	1.0	2.0	0.50	0.38	0.89	0.919	12	1.838
5 JB	160	2.3	4.6	0.25	0.41	0.93	1.572	15	3.144
6 MR	150	1.0	2.1	0.50	0.73	0.87	1.379	23	2.942
7 SG	150	1.6	3.4	0.30	0.32	0.98	0.969	9	2.067
8 RV	180	2.1	3.7	0.25	0.25	1.00	1.046	10	1.860
9 EL	150	2.1	4.5	0.25	0.35	1.00	1.390	11	2.965
Mean	161	1.6	3.2	0.36	0.42	0.93	1.202	2.393	0.89

PAIRED T-TEST									
Parameter					Treatment A versus Treatment B			Treatment A versus Treatment C	
					AUC	t-1/2	t-max	C-max	
					0.425 (0.646)				0.057 *
						0.100			0.047
							n.a.		n.a.
								0.202 (0.338)	0.197 *
									F Treatment B versus C = 0.209 (0.384)

* = corrected for difference in the administered sodium nitrite dose
n.a. = not applicable
() = results of volunteer 5 excluded

Appendix 18a**Table of Plasma Nitrate Concentrations**

Treatment A = Intravenous administration of 0.12 mmol NaNO₂ per mmol Hb to adult volunteers

Volunteer number	1 TN	2 MP	3 IS	4 DL	5 JB	6 MR	7 SG	8 RV	9 EL
Scheduled Time (hr:min)	Scheme Time (hr)	Actual Time (hr)	Nitrate (mg/kg)						
8.00	-1.00	-2.25	<2	-1.58	<2	-1.50	2.7	-1.85	<2
8.55	-0.08	-1.52	<2	-0.17	<2	-0.33	2.3	-0.17	<2
9.10	0.17	0.22	2.3	0.17	5.6	0.17	4.2	-0.42	<2
9.20	0.33	0.30	6.4	0.33	9.7	0.25	6.4	0.17	3.4
9.30	0.50	0.53	8.5	0.52	12.8	0.50	7.8	0.33	2.8
9.35	0.58	0.63	10.5	0.60	12.8	0.62	10.1	0.53	7.5
9.40	0.67	0.78	11.2	0.67	13.2	0.72	11.0	0.60	8.5
9.50	0.83	0.88	11.7	0.83	13.1	0.87	12.2	0.83	9.4
10.00	1.00	1.05	13.7	1.00	15.9	1.00	12.3	1.00	11.1
10.10	1.17	1.22	14.1	1.17	16.3	1.20	13.4	1.02	12.4
10.20	1.33	1.38	14.1	1.35	16.2	1.33	13.0	1.17	12.8
10.30	1.50	1.58	15.0	1.50	16.5	1.52	13.4	1.35	13.6
10.45	1.75	1.88	15.9	1.75	16.9	1.75	13.1	1.50	13.2
11.00	2.00	2.05	14.7	2.00	15.0	2.00	13.5	1.75	11.4
11.30	2.50	2.55	15.4	2.50	16.4	2.50	13.6	2.02	12.9
12.00	3.00	3.00	14.1	3.00	14.9	3.00	12.3	2.53	12.9
13.00	4.00	4.02	13.3	4.00	12.5	4.00	11.8	2.93	11.9
14.00	5.00	5.00	11.7	4.98	11.3	5.00	9.5	5.00	10.3
15.00	6.00	6.05	10.1	6.00	6.00	6.00	8.3	5.00	7.9
17.00	8.00	8.00	7.9	8.00	8.3	8.05	8.0	6.00	6.6
21.00	12.00	11.98	-	12.13	9.0	12.00	7.1	12.00	5.4
1.00	16.00	5.5	16.00	5.9	15.92	5.4	16.08	4.4	12.03
9.00	24.00	23.92	4.5	23.83	4.6	23.95	4.3	24.03	3.4
					<2		23.75	<2	24.00
								<2	24.02

Scheme Time and Actual Time are expressed as a time-interval in relation to the time of sodium nitrite administration
- = missing sample

**Appendix 18a Table of Plasma Nitrate Concentrations
(continued)** Treatment B = Oral administration of 0.12 mmol NaNO₂ per mmol Hb to adult volunteers

Volunteer number	1 TN			2 MP			3 IS			4 DL			5 JB			6 MR			7 SG			8 RV			9 EL					
	Scheduled Time (hr:min)	Scheme Time (hr)	Actual Time (hr)	Nitrate (mg/kg)																										
8.00	- 1.00	- 1.25	2.2	- 1.55	<2	- 0.83	3.2	- 1.08	<2	- 1.78	<2	- 0.92	<2	- 1.17	<2	- 0.92	<2	- 0.67	<2	- 0.38	<2	- 0.17	<2	- 0.42	<2	ND	ND			
8.55	- 0.08	- 0.03	<2	- 0.55	<2	- 0.08	2.9	- 0.77	<2	- 0.67	<2	- 0.25	5.4	0.25	3.2	0.25	6.4	0.25	8.1	0.25	10.1	0.50	10.4	0.50	7.1	0.25	7.1	0.25		
9.15	0.25	0.25	4.3	0.25	6.6	0.25	4.5	0.25	4.7	0.25	9.0	0.47	11.1	0.50	5.4	0.50	5.4	0.50	9.5	0.50	10.1	0.50	10.4	0.50	10.4	0.50	10.4	0.50		
9.30	0.50	0.50	9.5	0.50	9.4	0.50	9.2	0.48	9.0	0.47	11.1	0.50	11.1	0.50	5.4	0.50	5.4	0.50	9.5	0.50	10.1	0.50	10.4	0.50	10.4	0.50	10.4	0.50		
9.45	0.75	0.75	12.8	0.75	11.6	0.75	7.6	0.80	12.2	0.75	12.8	0.75	12.8	0.75	7.9	0.78	11.2	0.75	10.8	0.75	11.8	0.75	11.8	0.75	11.8	0.75	11.8	0.75		
10.00	1.00	1.00	14.0	1.00	11.8	1.00	9.5	1.00	12.4	1.00	13.0	1.00	13.0	1.00	8.8	1.02	12.6	1.00	12.2	0.98	16.5	1.25	13.5	1.25	13.8	1.25	13.8	1.25	13.8	1.25
10.15	1.25	1.25	14.8	1.25	12.9	1.25	11.7	1.25	13.5	1.25	13.6	1.25	13.6	1.25	10.0	1.30	13.4	1.30	11.2	1.50	11.2	1.50	13.5	1.50	13.5	1.50	13.5	1.50	13.5	1.50
10.30	1.50	1.50	15.5	1.50	13.9	1.50	12.2	1.50	14.3	1.50	13.3	1.50	13.3	1.50	13.3	1.50	13.3	1.50	13.3	1.50	13.3	1.50	13.3	1.50	13.3	1.50	13.3	1.50		
10.45	1.75	1.75	14.7	1.80	13.2	1.75	12.2	1.75	11.9	1.75	12.2	1.75	11.9	1.75	12.2	1.75	10.9	1.60	12.9	1.75	11.4	1.75	11.7	1.75	11.7	1.75	11.7	1.75		
11.00	2.00	2.00	15.2	2.03	13.4	2.00	12.5	2.00	13.7	2.00	14.2	2.00	10.9	2.02	12.4	2.00	13.0	2.00	12.2	2.00	12.2	2.00	12.2	2.00	12.2	2.00	12.2	2.00		
11.30	2.50	2.50	15.2	2.52	12.8	2.50	12.1	2.50	12.1	2.50	12.1	2.50	12.1	2.50	12.1	2.23	10.5	2.50	12.4	2.50	11.9	2.50	11.8	2.50	11.8	2.50	11.8	2.50	11.8	
12.00	3.00	3.00	14.0	3.00	12.3	3.00	12.1	3.00	11.4	3.00	12.2	2.92	10.2	3.00	11.5	3.00	11.1	3.00	11.5	3.00	11.1	3.00	11.1	3.00	11.1	3.00	11.1	3.00		
12.30	3.50	3.50	13.1	3.52	11.6	3.50	11.0	3.52	11.0	3.50	11.0	3.50	11.0	3.50	11.0	3.50	9.7	3.50	10.1	3.50	10.8	3.50	11.0	3.50	11.0	3.50	11.0	3.50	11.0	3.50
13.00	4.00	4.00	13.5	4.03	11.0	4.00	12.5	4.00	10.6	4.00	10.6	4.00	10.6	4.00	10.6	4.00	9.8	4.00	9.8	4.00	9.8	4.00	9.8	4.00	9.8	4.00	9.8	4.00	9.8	4.00
14.00	5.00	5.00	10.7	5.02	9.1	5.00	8.9	5.00	8.4	4.97	8.6	5.00	7.4	5.00	7.4	6.00	7.4	6.00	7.4	6.00	7.4	6.00	7.4	6.00	7.4	6.00	7.4	6.00		
15.00	6.00	6.02	10.0	6.02	8.6	6.00	9.3	6.00	6.8	5.98	7.9	6.00	7.4	6.00	7.4	6.00	7.4	6.00	7.4	6.00	7.4	6.00	7.4	6.00	7.4	6.00	7.4	6.00		
17.00	8.00	8.00	8.1	8.13	7.7	8.00	7.7	8.00	6.3	8.12	6.3	8.08	6.8	8.00	6.8	5.6	7.95	7.3	8.00	5.8	8.00	5.8	8.00	5.8	8.00	5.8	8.00	5.8	8.00	5.8
21.00	12.00	12.00	6.9	11.98	5.7	12.00	8.0	12.00	4.4	12.10	5.5	12.08	4.5	12.00	4.5	3.5	11.98	5.3	12.00	4.5	12.00	4.5	12.00	4.5	12.00	4.5	12.00	4.5	12.00	4.5
1.00	16.00	16.00	4.8	16.00	6.2	16.08	6.2	16.08	3.4	15.92	3.9	16.02	4.0	16.00	4.0	2.7	16.07	4.9	16.08	3.3	16.08	3.3	16.08	3.3	16.08	3.3	16.08	3.3	16.08	3.3
9.00	24.00	24.00	3.8	23.93	3.0	23.93	4.6	23.75	2.1	23.08	2.5	23.55	2.5	23.55	2.5	2.6	24.00	3.2	24.00	3.2	24.00	3.2	24.00	3.2	24.00	3.2	24.00	3.2	24.00	3.2

Scheme Time and Actual Time are expressed as a time-interval in relation to the time of sodium nitrite administration
- = missing sample

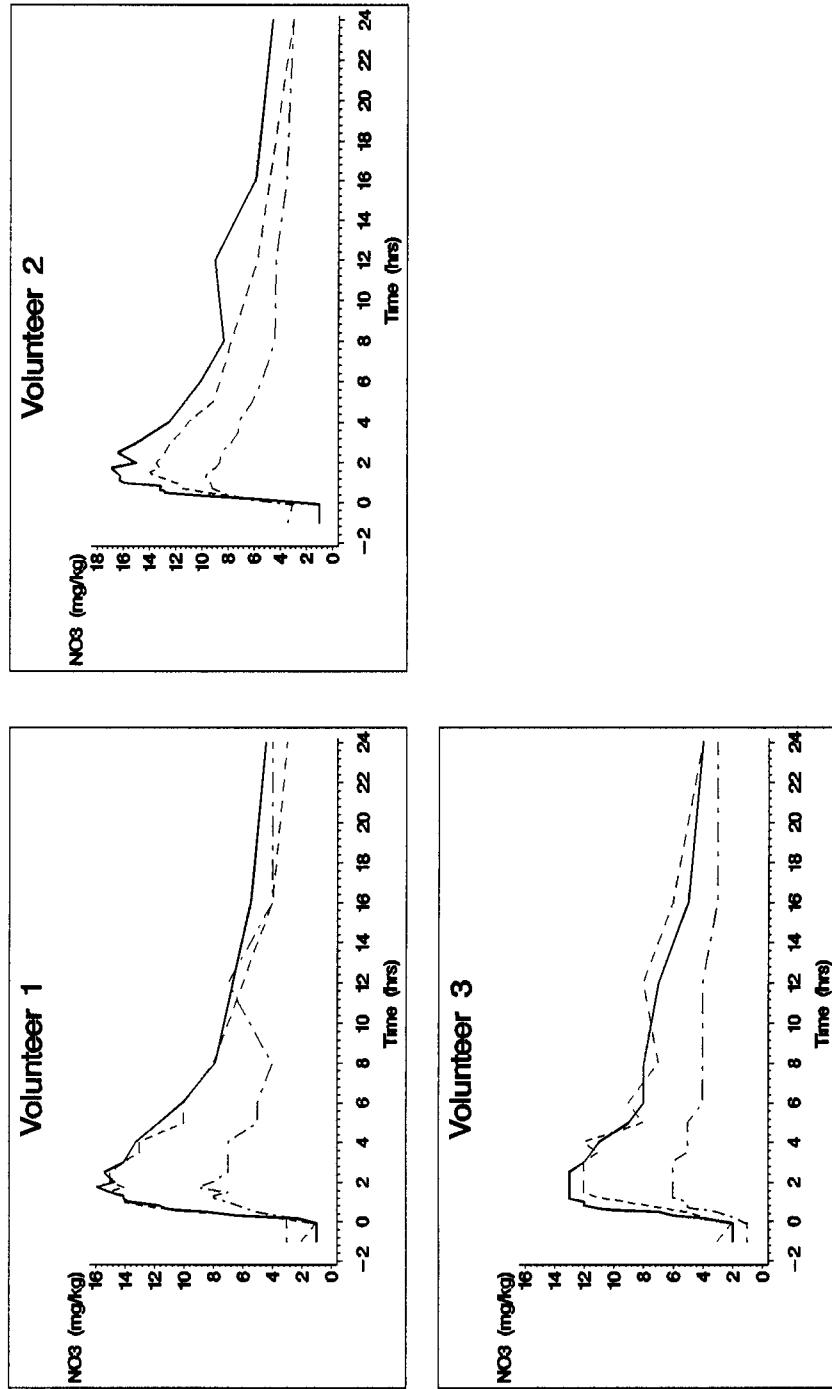
**Appendix 18a Table of Plasma Nitrate Concentrations
(continued) Treatment C = Oral administration of 0.06 mmol NaNO₂ per mmol Hb to adult volunteers**

Volunteer number	1 TN	2 MP	3 IS	4 DL	5 JB	6 MR	7 SG	8 RV	9 EL
Scheduled Time (hr/min)	Scheme Time (hr)	Actual Nitrate (mg/kg)	Actual Time (hr)	Nitrate (mg/kg)	Actual Time (hr)	Nitrate (mg/kg)	Actual Time (hr)	Nitrate (mg/kg)	Actual Time (hr)
8.00	-1.00	-1.55	3.2	-1.58	3.4	-3.00	<2	-1.87	<2
8.55	-0.08	<2	-0.12	3.0	-1.38	<2	-0.72	-0.42	<2
9.15	0.25	3.3	0.17	7.5	0.25	2.2	0.25	4.1	0.25
9.30	0.50	5.7	0.53	8.4	0.50	3.8	0.50	5.5	0.50
9.45	0.75	6.6	0.75	9.2	0.75	5.3	0.77	7.3	0.75
10.00	1.00	1.00	7.9	1.00	9.3	1.00	1.00	1.00	1.00
10.15	1.25	8.0	1.25	9.7	1.25	6.3	1.27	7.5	1.25
10.30	1.50	1.52	7.8	1.50	9.5	1.50	7.7	1.50	1.50
10.45	1.75	1.78	9.6	1.73	8.9	1.77	6.3	1.75	1.75
11.00	2.00	2.00	8.4	1.98	8.6	2.02	7.1	2.00	7.1
11.30	2.50	7.7	2.50	8.5	2.50	6.3	2.52	6.4	2.50
12.00	3.00	3.00	7.1	3.00	7.7	3.00	6.7	3.00	6.7
12.30	3.50	3.50	7.3	3.50	7.2	3.50	5.3	3.50	5.3
13.00	4.00	4.00	7.2	4.00	5.4	4.00	5.6	4.00	5.4
14.00	5.00	5.00	5.7	5.00	6.1	5.00	5.8	4.2	4.00
15.00	6.00	6.00	5.1	6.00	5.4	6.00	4.4	4.5	5.00
17.00	8.00	8.00	4.9	8.00	4.4	8.08	3.5	8.00	4.8
21.00	12.00	12.17	7.3	12.00	4.3	12.05	3.0	12.03	3.0
1.00	16.00	16.03	4.6	16.00	3.5	16.08	2.1	16.12	3.2
9.00	24.00	23.83	4.5	24.00	3.0	23.57	<2	23.75	2.3

Scheme Time and Actual Time are expressed as a time-interval in relation to the time of sodium nitrite administration

Appendix 18b Plasma Nitrate concentration as observed after single dose administration of sodium nitrite to adult volunteers
(continued)

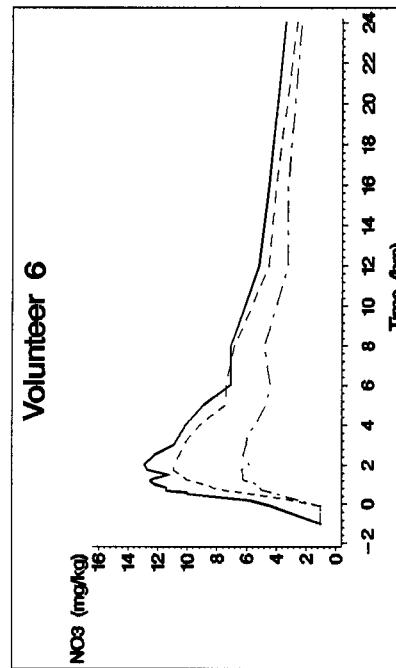
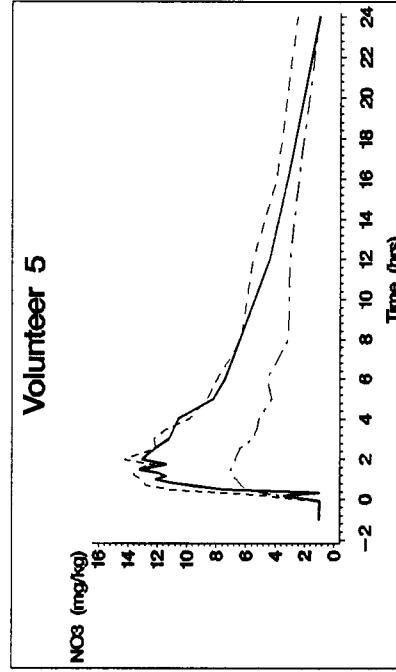
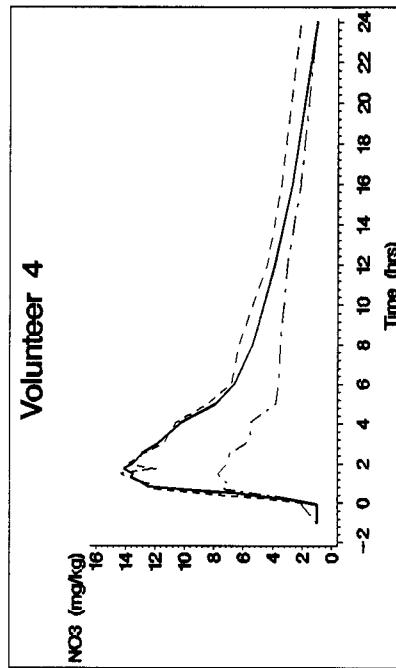
- (—) = Treatment A (intravenous administration of 0.12 mmol sodium nitrite per mmol Hb)
- (---) = Treatment B (oral administration of 0.12 mmol sodium nitrite per mmol Hb)
- (- - -) = Treatment C (oral administration of 0.06 mmol sodium nitrite per mmol Hb)



Appendix 18b
(continued)

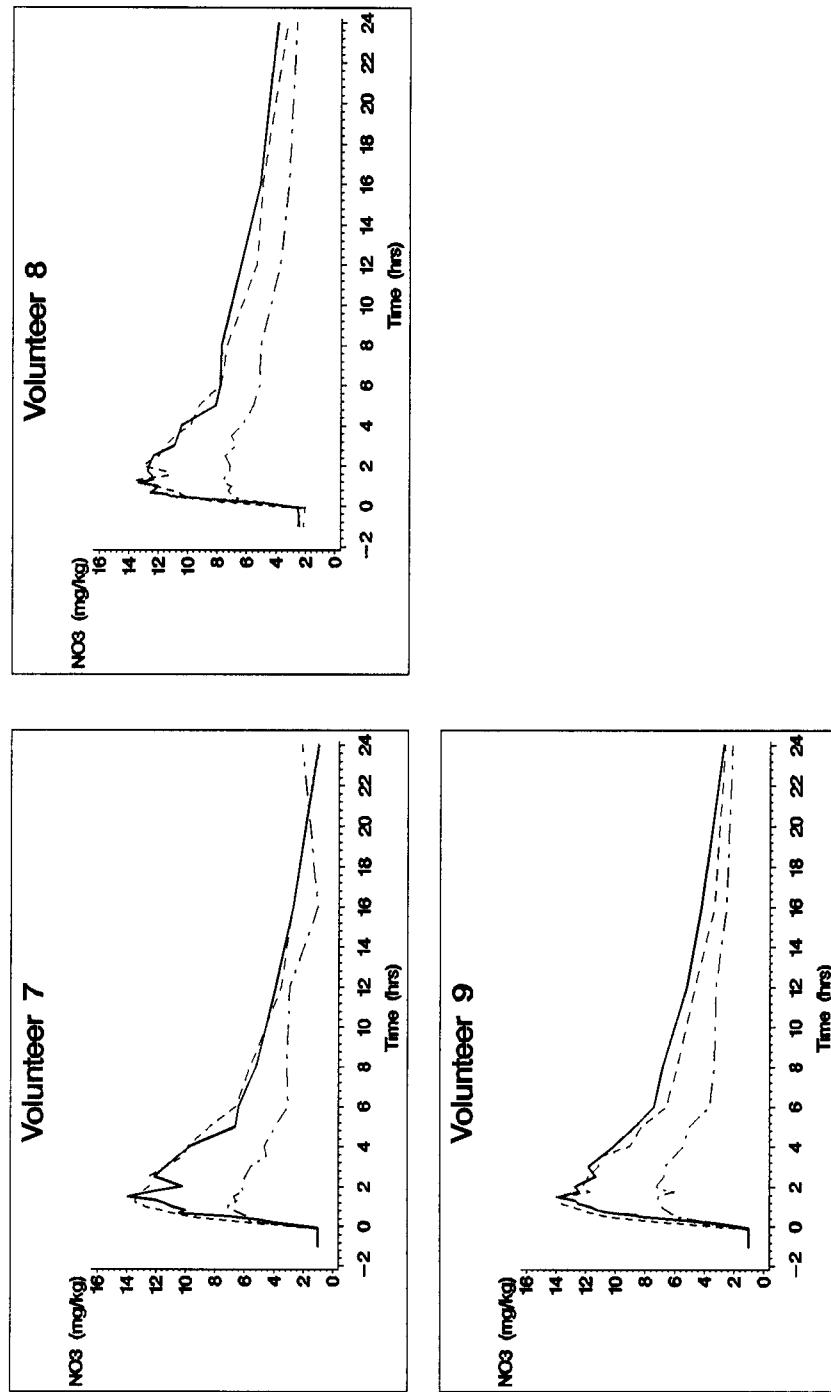
Plasma Nitrate concentration as observed after single dose administration of sodium nitrite to adult volunteers

- (—) = Treatment A (intravenous administration of 0.12 mmol sodium nitrite per mmol Hb)
- (---) = Treatment B (oral administration of 0.12 mmol sodium nitrite per mmol Hb)
- (- - -) = Treatment C (oral administration of 0.06 mmol sodium nitrite per mmol Hb)



Appendix 18b
Plasma Nitrate concentration as observed after single dose administration of sodium nitrite to adult volunteers
(continued)

(—) = Treatment A (intravenous administration of 0.12 mmol sodium nitrite per mmol Hb)
(---) = Treatment B (oral administration of 0.12 mmol sodium nitrite per mmol Hb)
(-·-) = Treatment C (oral administration of 0.06 mmol sodium nitrite per mmol Hb)



Appendix 19 Table of kinetic parameters derived from the Plasma Nitrate Concentrations

Treatment A					
Volunteer number	Nitrite dose (mg)	C-max (mg/kg) adjusted*	t-max (hrs)	t-1/2 (hrs)	AUC(0-24) (mg*hr/l) adjusted*
1 TN	350	13.9	12.7	1.88	10.1
2 MP	380	14.8	12.5	1.75	10.4
3 IS	290	11.2	12.4	2.50	10.1
4 DL	330	12.5	12.1	1.75	4.5
5 JB	310	12.1	12.5	1.50	6.3
6 MR	300	11.2	11.9	2.17	9.4
7 SG	300	12.5	13.3	1.50	5.5
8 RV	360	11.0	9.8	1.17	9.9
9 EL	290	12.4	13.7	1.50	7.7
Mean	323	12.4	12.3	1.75	8.2
					110.5
					109.4

Treatment B					
Volunteer number	Nitrite dose (mg)	C-max (mg/kg) adjusted*	t-max (hrs)	t-1/2 (hrs)	AUC(0-24) (mg*hr/l) adjusted*
1 TN	350	13.5	12.3	1.50	8.2
2 MP	380	11.8	9.9	1.50	6.3
3 IS	290	10.1	11.1	2.00	11.9
4 DL	330	12.7	12.3	1.50	5.2
5 JB	310	13.1	13.5	2.00	7.6
6 MR	300	9.2	9.8	1.75	6.8
7 SG	300	12.0	12.8	1.30	4.6
8 RV	360	11.2	10.0	1.25	6.9
9 EL	290	15.0	16.6	0.98	7.5
Mean	323	12.1	12.0	1.53	7.2
					105.4
					104.8

PAIRED T - TEST			
Parameter	Treatment A versus Treatment B	Treatment A versus Treatment C	Treatment A versus Treatment B
AUC	0.3631	0.1835 *	0.2797
t-1/2		0.1860	0.4455
t-max		0.0817	0.1679 *
C-max		0.5584	

Treatment C					
Volunteer number	Nitrite dose (mg)	C-max (mg/kg) adjusted*	t-max (hrs)	t-1/2 (hrs)	AUC(0-24) (mg*hr/l) adjusted*
1 TN	170	7.6	14.3	1.78	41.5
2 MP	190	7.6	12.8	1.25	9.3
3 IS	140	4.3	9.8	3.00	11.7
4 DL	160	6.1	12.2	1.50	5.5
5 JB	160	5.9	11.8	1.25	8.7
6 MR	150	4.6	9.8	1.75	8.1
7 SG	150	5.7	12.2	1.00	12.2
8 RV	180	5.3	9.4	1.25	5.5
9 EL	150	5.7	12.2	2.00	8.5
Mean	161	5.9	11.6	1.64	12.3
					51.4
					101.9

* = corrected for difference in the administered sodium nitrite dose
adjusted* = results adjusted to a standard dose of 320 mg sodium nitrite