

Annex B. Human studies with simulants

B.1. Introduction

Simulants can be used instead of CW agents for training and testing. Training simulants are typically used in CW training exercises in which members of the Armed Forces practise defensive drills designed to protect them from CW attacks. Those drills might involve setting up field detectors, decontaminating equipment, maintaining a clean environment in a building as well as donning personal protective clothing. Generally, training simulants are used to enable umpires, who preside over exercises, to determine if drills have been followed properly by the participants and if protective clothing is effective. During exercises simulants are dispersed in vapour or liquid form in much the same way a real CW agent would be dispersed.

There are two types of training simulants which serve this purpose: they might be termed inactive and active.

- Inactive simulants. These are simulants which when inhaled or fall on skin can later be detected on expired breath or in body fluids (blood or urine, usually). The amount detected can be used to gauge the degree of decontamination experienced. This type of simulant induces no physiological effect (hence "inactive") but has the same physical properties (volatility, density etc.) as the agent it represents. DMSO and Methyl Salicylate are examples of inactive simulants. Typically, human studies at Porton sought to establish if these simulants could be detected on breath or in body fluids after they had been inhaled or placed on the skin.
- Active simulants. These are simulants which impose some physiological penalty on exercise participants who do not follow drills correctly or who have faulty protective clothing. They are less toxic than CW agents. T4423 is an example of a substance considered as an active simulant and was investigated as an alternative to using CS in training exercises. Human studies with T4423 are covered in the riot control part of the survey report.

Testing simulants are not used in training. They are often used in laboratory, animal and human tests to investigate scientific aspects which apply to real CW agents. Testing simulants can be used in various ways.

- Skin penetration. The biomechanics of liquid CW agents penetrating human skin can be investigated with simulants. In the nerve agent part of the report it will be seen that TPP (a simulant for G agents) and phosphate or sodium ions were used instead of GB or VX to understand how these agents penetrate the skin.
- Miosis. Human studies of nerve agents sought to investigate the effect of miosis on military tasks. In some of them, as mentioned in the nerve agent part of the report, substances which induced miosis (such as physostigmine salicylate) were used instead of GB or VX.
- Pick-up trials. Inactive simulants were used to assess the risk of becoming contaminated when men operated in an environment previously contaminated by a persistent agent. These tests, referred to as pick-up trials, are outlined in the chapter on V agents and used DEP as a simulant for VX.
- Respirator testing. When Porton designed new respirators human studies were often carried out to check if they leaked. Latterly in the period covered by the survey, tests of the facelet (designed to protect the nose and mouth) were sometimes conducted with GB or CS. Halothane and Penthrane were investigated as alternative, and relatively harmless, simulants for these leakage assessments.

B.2. Human studies with training simulants

B.2.1. Dimethyl Sulphoxide (DMSO)

DMSO was first considered as a simulant in 1968 and tests involved animal work to study its effects. At that time a method to detect very small amounts of DMSO in body fluids remained to be found¹. By 1980 animal work had been completed, extensive literature on the medical effects of DMSO had been consulted and a method developed to measure DMSO in expired breath. A study was conducted to test the method², in which 12 volunteers took part. 50 mg of pure DMSO or DMSO diluted in water was placed on the forearm and covered with a watch glass. Breath samples were taken at about 45 minute intervals for 6 hours after DMSO had been applied. When a sample was taken, the volunteer held his breath for 15 seconds and exhaled into a tube.

- Three volunteers had pure DMSO placed on their arm, two had a 90% solution in water and seven had a 50% solution in water.
- The method was found to be capable of detecting very low concentrations of DMSO on expired breath and it was concluded that the method would be able to detect DMSO in breath following a skin contamination of about 4 mg.

Animal work and open literature suggested that DMSO applied to skin induced erythema. As the effect had not been fully investigated a short study was carried out in 1980 in which 8 volunteers participated³.

- Two volunteers had a 90% solution of DMSO applied to their forearm, confined by a plastic ring and a watch glass and left for one hour. Three and a half weeks later the same volunteers had a 50% solution of DMSO applied to the same site on the forearm. Erythema developed around hair follicles in these two volunteers after the application of 90% DMSO, fading over the course of the following day. After the second application of DMSO slight erythema developed under the edge of the plastic ring.
- Six other volunteers had a single application of 50% DMSO on the forearm. No erythema developed.

The study concluded that the development of erythema was related to the concentration of DMSO applied, rather than the dose. The next study with DMSO was carried out in 1983 when a new and improved technique to measure DMSO on expired breath had been developed⁴. The study tested this technique and also measured DMSO in the breath of people who had not had DMSO applied to their forearm⁵.

- Twenty volunteers took part, split into two groups, with each group participating in two tests separated by a week.
- The men had 50 mg or 100 mg of an 80% DMSO solution placed on their forearm and smeared with a spatula. To guard against an inadvertent inhalation of DMSO from the open flask from which it was applied, some men wore facelets.

¹ COSHE 49th meeting 22 Oct 68.

² Technical Note 450. Dimethyl sulphoxide as a CW agent intake simulant: estimation of dosage by expired breath analysis. Jan 81. [R]

³ Technical Note 460. Cutaneous manifestations following the administration of DMSO in guinea-pigs and man. Mar 81. [R]

⁴ COSHE 157th meeting 11 Jul 83.

⁵ Technical Note 642. Dimethyl sulphoxide as a CW agent intake simulant. Part 2: Further human exposures and measurements of dimethyl sulphoxide in breath samples from unexposed individuals. Oct 84. [UK R]

The amount of DMSO measured on expired breath was found to depend more on the individual than on the dose applied to the forearm. DMSO was measured on the breath of people who had not been exposed to DMSO: 60 members of the Porton staff and 56 volunteers (from No. 42 Survey Regiment RE stationed at Drayton Camp in Barton Stacey) took part. Each person was asked about their diet and any form of skin creams and ointments they used (all or any of which might affect the presence of DMSO). DMSO on the breath of these people was found to vary widely.

This work ended human studies with DMSO in the period covered by the survey. Evidently an improved technique for measuring DMSO on breath was developed. COSHE approved in October 1989 a study protocol to test this method in volunteers receiving an application of DMSO on their skin⁶.

B.2.2. Diethyl morpholino phosphoramidate (DEMPA)

DEMPA was considered as a simulant for VX in 1974⁷. DEMPA was excreted in urine after application to the skin. Animal work showed it to be of low toxicity by all routes and COSHE considered a proposal for a human study in February 1975⁸: up to 10 µl of DEMPA was to be applied to the face of volunteers whose urine would then be subject to gas chromatography analysis to detect the amount excreted. DEMPA had been used outside Porton at doses of 20 µl without any adverse effect.

COSHE approved this study but stipulated that DEMPA should be subjected to more chemical tests before the study began⁹. These tests continued for the remainder of 1975. COSHE consulted the Chester Beatty Research Institute¹⁰ who suggested that mutagenicity should be checked by using bacteria and the central toxicology laboratory of a commercial chemical company was contracted to perform the work¹¹. The company completed the tests (using the Ames bacterial method) and found no significant mutagenic activity. In reporting the work to Porton the company noted that the Ames test was able to predict the mutagenic activity of a compound to mammals with 90% accuracy¹².

In December 1975 COSHE, noting this satisfactory result, further deferred the human study with DEMPA until work had been conducted with chickens to investigate any possible neuro-toxic effect¹³. This work continued to the end of the summer of 1976¹⁴ and involved many animal species. In none was evidence of neuro-toxicity found after DEMPA had been given orally and no organ damage was observed¹⁵.

The human study started in late 1976 and continued into the spring of 1977. Twenty volunteers took part each having DEMPA placed on their facial skin and having samples of their urine tested. Details of the study are given below¹⁶.

- Two men had 0.2 µl applied, 3 men and 4 women had 1 µl, and 9 men and 2 women had 5 µl.
- No untoward symptoms were observed but the amount of DEMPA excreted subsequently in urine varied between individuals.

⁶ COSHE 184th meeting 19 Oct 89.

⁷ COSHE 92nd meeting 13 Mar 74.

⁸ COSHE 98th meeting 17 Feb 75.

⁹ COSHE 99th meeting 14 Apr 75.

¹⁰ COSHE 101st meeting 7 Jul 75.

¹¹ COSHE 102nd meeting 1 Sep 75.

¹² MC Proceedings. Letter from company to Porton. 14 Nov 75.

¹³ COSHE 104th meeting 8 Dec 75.

¹⁴ COSHE 107th meeting 14 Jun 76.

¹⁵ COSHE 106th meeting 10 Mar 76.

¹⁶ Request for permission to increase the dose of DEMPA applied topically to skin. Med/IT4010/471/77 22 Apr 77.

In April 1977 COSHE considered a proposal to increase the dose of DEMPA applied to the face. However, this was overtaken by a message from two doctors at the company who had conducted mutagenicity tests on DEMPA. They queried whether the Ames test was suitable for a compound like DEMPA and suggested that another test (called the Styles Cell Transformation test) should be used. In light of this, COSHE halted further human studies until the test had been applied¹⁷. As well as contracting the company to perform the test, Porton sent a sample of DEMPA to "US authorities" for testing¹⁸.

The results of these further tests were discussed by COSHE in January 1978. The toxicology laboratory of the company confirmed that DEMPA was mutagenic by the Styles test but had again proved negative by Ames. COSHE decided to end human studies with DEMPA, noting that nobody should be intentionally exposed to the compound¹⁹.

B.2.3. Salicylates

In 1969 Methyl Salicylate (MeS) was considered as a training simulant. It had been used previously as a testing simulant for VX, GD and H in various trials investigating the degree of pick-up contamination with men wearing full protective clothing. The proposal considered by COSHE sought permission to use MeS in a troop trial at a level which would ensure that each man might be subject to a maximum dose of 1 g falling on his skin. The proposal listed characteristics of MeS²⁰:

- MeS was rapidly absorbed through skin and induced progressive symptoms (headache, dizziness, sweating, thirst, nausea, diarrhoea) and, in large enough doses, convulsions and death;
- early symptoms, up to nausea, were produced by doses of about 12 g;
- MeS was used in the treatment of rheumatism, applied as 3-8 ml daily to the afflicted parts and covered with an airtight bandage;
- the oral LD50 in animals varied according to species from 700-2750 mg/kg;
- MeS can cause eye damage in doses of 0.1-0.5 ml.

COSHE agreed to the use of MeS in the troop trial but stipulated that the eyes should be fully protected²¹. Before the troop trial went ahead, human tests were carried out to check that MeS falling on skin could be detected subsequently in urine (N.B. in the pick-up trials conducted before 1969 this was not of relevance because men wore full protective clothing and the issue was how much MeS had been picked-up on their clothing).

The human tests applied 300 mg of MeS to the skin of volunteers in 5 mg drops²². The drops were left uncovered. Urine samples were taken 24 hours after application but no MeS was detected. Evidently the inference drawn from this result was that MeS could not be detected by the method used to test urine (rather than concluding that no MeS was present in urine). Consequently a proposal was made to COSHE to use Benzyl Salicylate (BS) to see if it was more detectable in urine. The toxicity of BS was poorly documented in the open literature but the few references available suggested it was relatively non-toxic: it was used in sun screen preparations in concentrations of 5-10%.

COSHE agreed to repeat the tests conducted with MeS with BS in late April 1969²³. No mention of the results of this work has been found. No reference to either BS or MeS

¹⁷ COSHE 113th meeting 26 Apr 77.

¹⁸ COSHE 114th meeting 30 May 77.

¹⁹ COSHE 117th meeting 23 Jan 78.

²⁰ COSHE proceedings. Troop trial with Salicylate Simulant. Med.B/IT4010/982/69 26 Mar 69.

²¹ COSHE 54th meeting 1 Apr 69.

²² COSHE proceedings. Proposal to use Benzyl Salicylate as simulant for GD instead of MeS.

Med.B/IT4010/1289/69 dated 28 Apr 69.

²³ COSHE 55th meeting 29 Apr 69.

appears in documents reviewed by the survey until 1986, when MeS was to be used as a testing simulant in field trials with men wearing full protective clothing²⁴.

B.3. Human studies with testing simulants

B.3.1. Simulants to induce miosis

Human studies were conducted with nerve agents to establish the degree of miosis they induced and the effect of that miosis on military performance. These studies are recounted in the nerve agent chapters. For some studies investigating the effect of miosis on performance, it was thought adequate to use a simulant which induced miosis but none of the other effects expected from nerve agents. By January 1975 approval had already been given by COSHE for physostigmine eye drops to be used as a simulant for miosis studies but the Institute of Aviation Medicine suggested pilocarpine eye drops should be used instead as they were shorter acting and less unpleasant²⁵. COSHE approved pilot trials to test pilocarpine nitrate eye drops.

In the event the trial considered various clinical eye drop preparations²⁶. Some were rejected as lasting too long or being too uncomfortable. Three were considered in the study, in different concentrations as shown below

No of men	Clinical eye drop preparation
2	0.25% physostigmine salicylate
5	2% pilocarpine nitrate
4	1% physostigmine sulphate
6	1.18% physostigmine salicylate
3	4% pilocarpine nitrate

0.25% physostigmine salicylate, 2% and 4% pilocarpine nitrate produced too little miosis and were too short lived to be of value. 1% physostigmine sulphate and 1.18% physostigmine salicylate were better, inducing miosis with a reduction in pupil area of about 90%. The sulphate was noted as being preferred.

B.3.2. Halothane and Penthrane

In the late 1970s it was noted that the respirator then used by the Services gave excellent protection against toxic agents but was unsuitable for long term wear in anticipation of a chemical attack as it imposed a physiological load on the wearer. Moreover, in a surprise chemical attack men may receive an incapacitating dose before they were able to don their respirators (assuming it took 60 seconds for a respirator to be put on and correctly adjusted). As a result Porton developed a simple oro-nasal mask (called the facelet) which was designed to be worn for long periods²⁷. The facelet was similar to a surgeon's mask and was made of absorbent material.

It was acknowledged that the facelet gave significant, albeit incomplete, protection. Work was carried out to investigate the degree of protection it afforded. During development, the protection given by facelet designs was investigated in human studies with GB, the details of which are given below²⁸.

- Three volunteers took part, two wearing the facelet along with a pair of goggles and the other being unprotected.

²⁴ COSHE 171st meeting 24 Nov 86.

²⁵ COSHE proceedings. Permission to use pilocarpine as a miotic. Med/IT4010/1207/75 dated 29 Jan 75.

²⁶ Technical Note 232. Short acting miotics as a model for nerve agent miosis. May 75. [R]

²⁷ Technical Paper 226. The facelet: a low efficiency oro-nasal mask for protection against surprise CW attack. Jul 77. [R]

²⁸ ibid (Technical Paper 226).

- The three men were exposed to GB at 5 mg.min/m³ (t = 20 minutes) and the degree of ChE inhibition was measured one hour after exposure as a means of assessing the protection afforded by the facelet.
- ChE inhibitions in the two men who wore the facelet varied from 0-5.7% (RBC, plasma and whole blood ChE were measured). The unprotected man had his ChE depressed by 14.3-21.1%.

The tests were deemed inconclusive but good enough to warrant further work. Some of this work would test GB penetration in studies with dummy heads.

However, in February 1977 other ways of checking facelet leakage, which did not require the use of GB, were conceived. These centred on the use of halothane and penthrane. Halothane could be detected by smell and penthrane could be detected in blood if it were inhaled. The use of both was considered by COSHE in February 1977²⁹. Halothane will be described first.

Proposals for human studies with halothane sought to determine the minimum concentration that could be detected by smell (presumably, this concentration might be used to check if the facelet leaked). Volunteers would enter a chamber containing only air and over the course of an hour the concentration of halothane would be slowly increased to 30 ppm. The time (and therefore concentration) that volunteers smelled halothane would be recorded. Advice was obtained from consultant anaesthetists.

- A consultant at the University College Hospital noted that operating theatre atmospheres frequently contained 15-20 ppm of halothane. Patients undergoing surgery might receive 500 ppm for 3-4 hours. Impairment of consciousness might be expected at 400 ppm. The consultant regarded a single exposure of 0-30 ppm over the course of an hour "perfectly safe, although repeated exposures might give cause for concern".
- A consultant for the Salisbury Group of Hospitals endorsed this view but thought that halothane was unlikely to be detected at a concentration below 50 ppm. Using 50 ppm, rather than 30 ppm, as the maximum concentration "would still be safe".
- COSHE, in reviewing these comments, decided that the human study should follow the procedure suggested in the proposal but the final concentration of halothane should be 100 ppm and it should be achieved at the end of a period of exposure of 3 hours. Volunteers who took part in the study would complete psychomotor tasks to measure the effect of halothane on their performance.

Volunteers were exposed to halothane in this way during the summer of 1977. The performance of the volunteers was unaffected by halothane but the concentration of halothane that could be detected by smell was not clearly established. Permission was sought from COSHE to increase the maximum concentration of halothane to 500 ppm. This was rejected because COSHE noted that consciousness might be impaired at 400 ppm³⁰.

After this meeting Porton sought further advice from consultant anaesthetists on the use of halothane. Discussions continued into March 1978 but no firm proposal for more studies was made. No reference to further work on halothane has been found.

At the time it was being considered as an alternative to GB for assessing facelet leakage penthrane was used as an analgesic by midwives in concentrations of about 3000 ppm. The proposal for the human study sought permission to expose volunteers for one hour at a concentration of 20 ppm. However, it was thought that if the facelet gave good protection a higher concentration ought to be used to ensure enough penthrane was inhaled so as to be

²⁹ COSHE proceedings. Special meeting 8 Feb 77.

³⁰ COSHE 116th meeting 31 Oct 77.

detected subsequently in blood. Accordingly, COSHE approved exposures from 20-100 ppm over the course of an hour.²⁹

This work was carried out in 1977 with some volunteers wearing facelets and others unprotected³¹. Evidently not enough volunteers were available for the work and the possibility of seeking volunteers from among the Porton staff was suggested. This was rejected by the MC as it thought that it was "questionable whether [Porton] staff could be considered bone fide volunteers for experiments at Porton under the terms of the Helsinki Declaration"³².

Work assessing the degree of facelet leakage by using penthrane continued into 1978. Studies suggested that the "facelet gave a protection factor of about 90%" and the length of exposure to penthrane to test the protection was proposed to be increased to 2 hours³³. COSHE approved this extension but stipulated that only volunteers who wore facelets were to be exposed for 2 hours to a concentration of 100 ppm. Volunteers who were exposed without facelets (effectively as controls) would only undergo an exposure of one hour and to a concentration of only 50 ppm³⁴. Facelet leakage was tested in this way in 1978 and 1979³⁵. A further facelet leakage trial was conducted in 1984³⁶.

³¹ Experimental Log MPG 74.

³² MC proceedings. Proposal to expose CDE staff to penthrane (undated, but the position on the file suggests the MC considered the proposal in the summer/autumn of 1977).

³³ COSHE 118th meeting 6 Mar 78.

³⁴ COSHE 119th meeting 5 May 78.

³⁵ Experimental Logs MPG 74, MPG 75, MPG 119.

³⁶ Experimental Log MPG 84.