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Immediate post-dosing paralysis following severe Soman and VX toxicosis in guinea pigs

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ABSTRACT: There have been numerous studies of the central nervous system (CNS) involvement in organophosphate (OP) poisoning showing *status epilepticus* and/or 'electrographic seizures'. Brain damage has been demonstrated as 'neuronal necrosis' primarily in the cortex, thalamus and hippocampus. To the authors' knowledge there have been no reports of partial/total paralysis following close upon OP exposure although delayed paralysis has been reported. This report summarizes the immediate, OP induced paralytic events recorded in guinea pigs during development of the Canadian reactive skin decontaminant lotion (RSDL®).

As part of the development work, supra-lethal cutaneous doses of OP were applied to large numbers of guinea pigs followed by decontamination with the RSDL® or predecessor lotions and solvents. Soman (pinacolyl methylphosphonofluoridate; GD) challenges were applied to 1277 animals and S-(2-diisopropyl-aminoethyl) methylphosphorothiolate (VX) challenges to 108. The classic sequence of clinical signs — ptyalism, tremors, fasciculations, convulsions, apnea and flaccid paralysis before death — was seen in the 658 animals that died and in many of the survivors. Eighty-four of 688 survivors of GD and 4 of 39 survivors of VX showed random paralysis of various distal regions following recovery from an insult which produced convulsions and/or flaccid paralysis. Because the experiments were designed to assess the decontamination procedures, there were no apparent relationships between the amounts of OP applied and the sequellae recorded. The observations of paralysis were also incidental to the prime focus of the experiments. Because of this, only ten animals paralysed following GD exposure were examined for histological effects. The pathologist diagnosed 'encephalomalacia' and 'focal necrotic lesions' in the cerebral cortex and 'focal necrotic lesions' in one spinal cord. Of the 84 guinea pigs paralysed after GD challenge, one was not decontaminated and the decontaminants used on the remainder were sufficiently varied that there appeared to be no relationship between the type of decontaminant and the resulting paralysis. Copyright © 2005 Crown in the right of Canada. Published by John Wiley & Sons, Ltd.

KEY WORDS: organophosphates; nerve agents; Soman; VX; brain damage; paralysis; guinea pigs

Introduction

There have been numerous studies of the central nervous system (CNS) involvement in the etiology of organophosphate (OP) poisoning. These have been summarized in several articles and textbooks (Anon., 1970; Baron, 1981; Sidell, 1997; Rump, 1999; Romano *et al.*, 2001). Romano *et al.* (2001) provide a referenced review of previous texts. Some human cases have been described (Baron, 1981; Szinicz *et al.*, 1999). 'Severe functional deficits in the primary sensory neuron . . .' have been described in cats (Goldstein, 1985a,b; Goldstein *et al.*, 1987). However, despite recurring references to CNS damage from OP poisoning (Bidstrup *et al.*, 1953; Bidstrup and Bonnell, 1954; Abou-Donia *et al.*, 1980, 2002; Baron, 1981; Koelle, 1981; Petras, 1981, 1984; McLeod *et al.*, 1984; Shih *et al.*, 1991; Ballough *et al.*, 1998; Lundy, 1999; Rump, 1999; Wood and Tattersall, 1999), to the authors' knowledge there have been no reports of paralysis following within 24 h of OP exposure.

The clinical signs of OP poisoning vary in the rate of onset, severity and duration according to the specific chemical involved, the route of administration and other factors (Baron, 1981; Koelle, 1981; Shih *et al.*, 1991). Immediate effects such as excess salivation (ptyalism), tremors, convulsions and respiratory failure may be incapacitating or lethal and, in survivors, long-term CNS effects can continue for extended periods up to the lifetime of the subjects. Delayed neurotoxicity is a common factor in chronic as well as acute organophosphate poisoning. The delayed effects begin to appear after extended periods in animals that appear unaffected immediately after exposure. The susceptibility differs between species (Baron, 1981). Man is affected, along with some non-human primates, domestic chickens, dogs, cats and ruminants. Laboratory species, including the guinea pig, tend to be refractory, susceptible under specific conditions or react in an atypical manner. In these delayed responses, the descriptions are of a progressive distal degeneration of the axons resulting in incoordination and paralysis.

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Brain damage has been reported in soman (GD) poisoning (Koelle, 1981; Petras, 1981, 1984; McLeod *et al.*, 1984; Shih *et al.*, 1991; Ballough *et al.*, 1998; Wood and Tattersall, 1999), in sarin (GB) poisoning (Abou-Donia *et al.*, 2002) and in poisonings with other OPs (Bidstrup and Bonnell, 1954; Aboui-Donia *et al.*, 1980). Both GD and sarin (GB) produce functional deficits in the cat (Goldstein, 1985a,b). GD can cause axon degeneration in the rat brain (Petras, 1981, 1984) which can be seen histologically using '... specialized neuroanatomical research stains ...' and '... will escape early detection by the use of conventional methods.' The effects of GD exposure apparently do not resemble those of experimental fetal hypoxia in monkeys or those resulting from delayed neurotoxicity in tri-ortho-cresylphosphate poisoning. The damage could be sparse to massive and has been found in animals that showed only mild clinical signs. The brain damage reported in GD poisoning has been associated with *status epilepticus* (Ballough *et al.*, 1998), epileptic type seizure activity (McLeod *et al.*, 1984; Shih *et al.*, 1991; Wood and Tattersall, 1999) and/or '... electrographic seizures' (Wood and Tattersall, 1999). The brain damage has been reported as 'neuronal necrosis' primarily in the cortex, thalamus and hippocampus (McLeod *et al.*, 1984). Similar effects have been reported following GB poisoning (Abou-Donia *et al.*, 2002). These studies in rats included sampling of the cerebellum, brain stem and spinal cord where no lesions were reported. However, reference is made in one paper (McLeod *et al.*, 1984) to '... occasional paralysis following an asymptomatic time interval of 7–14 days'. Evidence of axon and terminal degeneration in many regions of the CNS has been reported in soman intoxicated rats (Petras, 1981), cats (Koelle, 1981; Petras, 1981) and monkeys (Petras, 1984). Peripheral nerve damage (Cavanagh, 1961) and functional deficits (Goldstein, 1985a,b) have been reported in cats following OP exposures. Delayed paralysis has been reported in humans (Bidstrup and Bonnell, 1954). Immediate, distal paralyses are not mentioned in any of the above. As a complicating factor, one author refers to the problems encountered in studying the seizures because of the very steep dose–toxicity relationship involved (Wood and Tattersall, 1999).

Between 1987 and 1991, during the development of the Canadian reactive skin decontaminant lotion (RSDL®), a large number of guinea pigs were exposed to massive, cutaneous doses of GD and VX followed by decontamination with the RSDL® or predecessor lotions and solvents. Many of these animals exhibited the classic progression of clinical signs for nerve agent poisoning (Sidell, 1997; Romano *et al.*, 2001): namely, ptyalism, tremors, fasciculation, convulsions, apnea and flaccid paralysis before death. Many died but many also 'recovered'. A number of these 'recovered' survivors showed varying degrees of paralysis in different areas of the body. This report is intended to record and summarize the paralytic events recorded (as incidental observations) during development of the RSDL®.

Materials and Methods

Chemicals

Pinacolyl methylphosphonofluoridate (Sarin, GD, MW 182, CAS# 96-64-0) and O-ethyl S-(2-diisopropyl-aminoethyl) methylphosphorothiolate (VX, MW 268, CAS# 50782-69-9) were synthesized and purified by Mr A. Hansen at DRDC Suffield. The purity of the chemicals was tested on a weekly basis by gas chromatography/mass spectrometry and nuclear magnetic resonance. All chemicals were at least 96% pure. The LD_{50} values used in these studies were 2.6 mg kg^{-1} and 80 µg kg^{-1} for the percutaneous application to depilated guinea pigs of GD and VX, respectively (McDermot *et al.*, 1967; Connolley-Mendoza, 1987).

The RSDL® is a patented (Bannard *et al.*, 1991) preparation intended for skin decontamination of chemical warfare agents and other similar poisons. The lotions used in these studies and the decontamination potency have been described in previous reports (Bide *et al.*, 1988a,b, 1989, 1992; Armour *et al.*, 1989; Bide and Risk, 1991; Sawyer *et al.*, 1991). The RSDL® is a formulation of potassium 2,3-butanedione monoximate (KBDO) in a solvent — polyethylene glycol monomethylether (MPEG) of 550 nominal molecular weight. In addition, polyethyleneglycols (PEG) of various molecular weights, all of formulary grade, were used as the solvents in some of the decontamination studies.

All microliter doses of agent were dispensed using Western Model 800 positive displacement micro-volume dispensers (VWR Scientific, San Francisco, CA).

Animals

Male albino guinea pigs, *Cavia porcellus*, virus free, Hartley strain [CRL(HA)BC], 250–350 g body weight were purchased from Charles River Canada, St. Constant, Que. Housing and husbandry have been described in detail (Bide *et al.*, 1988a,b). All animals were acclimatized for at least 7 days in the vivarium at DRDC Suffield before use. When used, most animals were between 450 and 750 g body weight.

Decontamination Trial Procedure

All trial procedures were those used routinely in this laboratory in the decontamination studies intended for the development and validation of the RSDL®. Full details

including the numbers of mock exposed and control trials have been published elsewhere (Bide *et al.*, 1988a,b, 1989, 1992; Armour *et al.*, 1989; Bide and Risk, 1991; Sawyer *et al.*, 1991). Some early trials will have had minor deviations that should not have affected the results described in this report. The procedures were controlled and optimized to provide the most stable responses in the decontamination trials. Thus, the agent applications were made as a single drop at a single site to control and minimize the skin area contaminated. Similarly, the depilation procedure was as constant as possible to control and minimize the effects of this process upon the skin permeability and agent absorption.

During chemical exposure and subsequent treatment, the animals were immobilized in a stainless steel restrainer that was developed at DRDC Suffield (Connolley-Mendoza, 1987).

Sixteen to 48 h before chemical exposure, the backs of the subject guinea pigs were carefully shaved and depilated by application of Neet® depilatory cream (Rose scented Neet® was used exclusively because no skin emollients, oils or other modifying materials are added to this form of Neet®). After 20–30 min exposure to the depilatory (the time was reduced in the middle of the work to reduce the skin effects of the depiliatory), the animals were washed in running, tepid tap water and dried with paper towels. Vigorous rubbing was avoided. The animals were housed, individually, in stainless steel cages with mesh floors.

On the morning of the day of exposure, day 0, the animals were weighed, randomly distributed into experimental groups and placed in restrainers. At time 0, the prescribed dose of GD or VX was applied as a single drop on the middle of the back. After 55 s, decontaminant (0.7 ml) was applied to the target area from a polyethylene tuberculin syringe, taking care to cover the entire surface contaminated with the agent. The decontaminant was gently rubbed on the skin for 20 s with the syringe barrel to ensure full contact between the decontaminant, skin and remaining agent (all of the work described preceded the introduction of the current applicator sponge (Burcyk, 1997)). After 1 h, the decontaminant was wiped off using surgical gauze and, then, three 1 ml volumes of decontaminant were rapidly applied and wiped off. Each animal was washed, first with tepid tap water, then with 0.5% (v:v) Savlon® disinfectant solution and then washed again with tap water. After drying with paper towels, the animals were returned to their individual cages. The animals were watched for clinical signs of poisoning throughout exposure, decontamination and the immediate post-treatment periods. They were examined daily for 3 days thereafter, and were weighed on days 1, 2, 3 and on the two following Monday mornings. The survivors were killed on day 24.

The experimental protocols included mock exposed, control and blank trials, none of which caused any pathology or neuropathy.

Histopathology

The studies reported were focused upon the decontamination potency of the RSDL® so that histology was not done routinely on affected animals. The paralyses reported in this paper were incidental observations. The histological samples that were taken were from curiosity rather than a systematic attempt to define the tissue damage responsible for the paralytic events.

Complete brains and spinal cords and tissue samples of heart, spleen, liver, kidney and muscle and were taken from ten paralysed animals. The tissues were fixed in 10% formalin and shipped to the Department of Pathology, Western College of Veterinary Medicine, Saskatoon, Saskatchewan for processing and examination. The samples were imbedded, stained according to standard procedures with hematoxylin and eosin and sectioned prior to examination by light microscopy.

Results

Effects of GD

The doses of GD applied to the guinea pigs (Table 1) were intended to produce a 50% lethal effect following application of the decontaminant of the day. As the potency of the decontaminant increased, the doses increased accordingly. Consequently, the doses varied from 0.82 to 73.6 µl GD or 0.5 to 24 LD_{50}. The average dose was 15.4 µl or 8.4 LD_{50} of GD. As a result, there were no apparent dose-response relationships among the various groups of guinea pigs — dead, alive, affected and unaffected. The doses and ranges (Table 1) were statistically similar (F-test: $p < 0.05$).

Of the 1277 guinea pigs exposed to GD in these studies (Table 1), 1265 were decontaminated after about 1 min exposure (55–60 s according to the immediate experimental protocol applied) and then again after 1 h with the same decontaminant. The 14 others were part of an experiment to check the percutaneous toxicity of GD under the experimental conditions employed and were decontaminated after 1 h with soap and water only. Forty-six per cent of the guinea pigs exposed to GD succumbed to the nerve agent exposure. All of the mortalities showed the classic signs of GD toxicosis and were considered to be the result of nerve agent poisoning. The affected animals expressed, progressively, the usual sequence of clinical signs associated with organopho-

The procedure was originally developed for studies of HD decontamination. The Savlon® disinfectant was applied to reduce infection of the HD burns. The procedure continued as a routine process.

Table 1. Incidence of clinical signs in guinea pigs poisoned with GD

Treatment	Number of guinea pigs	Per cent in populations	Statistics for dose of GD (LD_{50})			
			Mean	SD	Min	Max
Total number	1277	100	8.4	±4.3	0.5	24
Dead	589	46	8.7	±4.1	2.0	24
Alive	688	54	8.1	±4.4	0.5	24
Animals alive						
Unaffected	340	49	7.1	±4.0	0.5	24
With toxicosis	348	51	9.1	±4.6	0.5	24
Affected survivors	348					
Ptyalism	332	95				
Tremors	108	31				
Convulsions	42	12				
Prostrate (flaccid)	99	28				
White tears	45	13				
Paralysis	84	24	8.8	±4.0	2	24
Where paralysed	84					
All quarters	31	37				
One side right	2	2				
One side left	1	1				
Front both	7	8				
Left only	4	6				
Right only	4	7				
Hind both	19	23				
Left only	2	2				
Right only	6	8				
Reflexes	8	9				
Decontaminant applied to paralysed guinea pigs						
Total paralysed	84	100				
Soap and water	1	1.2				
PEG only	14	17				
PEG KBDO	10	12				
MPEG only	11	13				
MPEG KBDO	48	57				

sphate poisoning, namely, ptyalism, tremors, convulsions, prostration (flaccid paralysis), apnea and death. Thirteen per cent also responded with opaque white tears in the later stages of the toxicosis. All of those affected had a clinical sign recorded before 1 h and, generally, the sequence was completed or in remission within 3 h.

For the animals listed as unaffected in Table 1, no clinical signs were observed. Of the survivors of GD challenges, 51% (348 animals) showed clinical signs of nerve agent toxicity with varying severity and either recovered or were 'recovering' when terminated for humane reasons (*vide infra*). The paralysed animals were all placed in this survivor group. The incidence of paralytic effects from GD (Table 1) was 24% in the animals that survived the poisoning and showed clinical signs. Of the total number of affected animals — including the mortalities — the incidence was 8.9%.

The paralysis was not related to the decontaminant used. Paralysis was observed with all of the five decontaminants applied (Table 1). Two polyglycol solvents were used with and without KBDO. In one case neither solvent nor active ingredient (KBDO) were applied.

To be scored as paralysed, an animal had to survive the toxic insult either to the end of the working day or, if the paralysis was not evident on that day, to the following morning. All paralysed animals had been in convulsion, prostration or flaccid paralysis and were recovering. Recovering, in the case of paralysed subjects, was defined as an animal conscious, responsive but unable to move various portions of the body. If food and water were placed within reach, many of the paralysed subjects would eat, drink and continue adjusting to the paralysis. In a few, more severe cases, the animals were breathing, sternally recumbent, unresponsive to external stimuli and would neither eat nor drink. In these cases, respiratory function, heart beat and reflexes could be established but mental awareness appeared absent.

The paralytic responses varied in the area and extent of the body affected (Table 1). The largest group (37%) were paralysed below the neck, i.e. all limbs paralysed. The second largest group had both hind legs paralysed. Smaller numbers had only one limb affected. Affected limbs were stiff, rigid and immobile. Paralysis of just paws or lower limbs was not observed. When the paralysis was first observed, some animals were allowed to continue for the 24-day observation period in the hope that remission might occur. As there was no improvement in these animals, all subsequent cases of paralysis

Table 2. Incidence of clinical signs in guinea pigs poisoned with VX

Treatment	Number of guinea pigs	Per cent in populations	Statistics for dose (LD_{50})			
			Mean	SD	Min	Max
Total number	108	100	23.7	±8.1	4	36
Dead	69	64	23.5	±9.0	4	36
Alive	39	36	24.1	±6.2	16	36
Animals alive						
Unaffected	13	33	20.6	±4.9	16	32
With toxicosis	26	67	25.9	±6.0	16	36
Affected survivors	26					
Ptyalism	26	100				
Tremors	6	23				
Convulsions	4	15				
Prostrate (flaccid)	4	15				
White tears	0	0				
Paralysis	4	15				
Mortalities	69					
Ptyalism tremors	69	100				
Convulsions/prostrate	69	100				
White tears	30	43				

All four paralysed guinea pigs were decontaminated with RSDL.

were terminated once the condition was recognized. The incidence of paralysis in the different body regions (Table 1) did not follow any pattern but appeared random. A chi square test of the incidence table indicated that the frequencies were similar ($p > 0.05$).

In the ten paralysed animals from which histology samples were obtained, the common histological finding was single or multi-focal damage in some area of the cerebral cortex. The veterinary pathologist described the changes as 'encephalomalacia'. The pathologist wrote of his findings in paralysed guinea pig 7 '— in all grey matter of the brain there are many darkly basophilic, shrunken neurons without any detectable glial reaction. In addition, there are extensive malacic lesions in the cerebral cortex, in which many of the neurons are shrunken, with strongly acidophilic cytoplasm and pyknotic nuclei. Severe gliosis, both focal and diffuse, and perivascular lymphocytic cuffing, accompany these changes. In severely affected areas there is vacuolation of the neutrophil of the granular layer and the overlying meninges are infiltrated with lymphocytes. The pyriforme lobe and the adjacent gyri are most severely affected and the lesion is bilaterally symmetrical . . .' In summary: — 'Definite lesions of cerebral cortical malacia were found in guinea pig 7. This is a definite premortem lesion . . . very similar, both in type and distribution, to lesions described in rats . . . (McLeod *et al.*, 1984). Diagnosis — poliomalacia of cerebral cortex with gliosis and perivascular cuffing'. Of another two brains the pathologist wrote of the background changes '. . . shrunken hyperchromatic neurons are found in all areas of the grey matter: some meningeal blood vessels contain increased numbers of neutrophils'. In addition to background changes '. . . contracted, basophilic neurons are particularly common in the cerebral cortex, tending to have laminar distribution in the middle of the granular layer . . . these changes (shrunken, hyperchromatic neurons) are very marked in the cerebral cortex at the midgranular layer. . . . Diagnosis – laminar poliomalacia'. For two other cases: — in addition to the background changes '. . . there is a single focus of astrogliosis located in the corpora quadridemia . . .' and '. . . there are scattered foci of vacuolation in the brainstem and cerebellar white matter — Diagnoses — Focal gliosis and vacuolation of the brain'.

Effects of VX

In all, 108 guinea pigs were exposed to VX in these studies (Table 2). All were decontaminated with RSDL after about 1 min exposure following the same procedure used with GD. Of the 108, 36% survived the VX challenge. All of the mortalities were considered to be the result of nerve agent poisoning and generally showed the classic signs of the organophosphate toxicosis. Of the survivors, 67% showed the clinical signs of VX toxicity and survived the insult. Four animals showed paralysis. The incidence of paralysis was 15% in animals that survived and showed clinical signs. Of the total number affected, including mortalities, the incidence was 4%.

The observed sequence of clinical signs was similar to that seen with GD albeit that the onset of signs was delayed – up to 2 h — and the times-to-death were proportionally longer. Among the affected survivors, ptyalism was always observed and tremors were occasionally seen.

Because the VX studies were done early in the sequence of experiments, the paralysis was not recognized

as a normal sequella and no histology was done on VX paralysed guinea pigs. Also, because of the structure of the experiments, there was no relationship demonstrated between dose of VX and the clinical signs observed (Table 2).

Discussion

To the authors' knowledge, this is the first formal report of partial/total paralysis as part of the immediate sequellae to organophosphate poisoning and the first report of brain damage in guinea pigs following organophosphate poisoning. The classical sequence of clinical signs — ptyalism, tremors, convulsions, flaccid paralysis, apnea and death — has been well documented in several texts (Anon, 1970; Sidell, 1997; Rump, 1999; Romano *et al.*, 2001). There is no mention in these texts of immediate paralysis in the survivors of acute poisoning. Delayed necrotic damage in the brain following organophosphate and nerve agent poisoning has been recognized for a long time and studied in several species (Koelle, 1981; Petras, 1981, 1984; Wood and Tattersall, 1999). Functional deficits have been reported (Goldstein, 1985a,b). One early paper (McLeod *et al.*, 1984) refers to paralysis occurring 7–14 days following exposure to organophosphates as an occasional outcome of 'delayed neurotoxicity'. Cats with functional deficits (Goldstein, 1985) received GD or GB either as a high dose (1 mg kg^{-1}) with atropine and physostigmine therapy or as lower doses repeated over several days. In separate studies, similar brain damage has been reported following both GD and GB challenges in rats (Abou-Donia *et al.*, 1980, 2002; Petras, 1981, 1984; McLeod *et al.*, 1984). In a study specifically intended to demonstrate the brain damage by GD in rats, no lesions were found in the brain stem, spinal cord or sciatic nerve (McLeod *et al.*, 1984). Delayed degenerative and necrotizing changes in the central nervous system and spinal cord have been reported in cases of human organophosphate exposure (Cavanagh, 1961; Abou-Donia *et al.*, 1980) and delayed paralysis has been reported in humans (Bidstrup and Bonnell, 1954). In the rat studies with GD and GB (Abou-Donia *et al.*, 1980, 2002; McLeod *et al.*, 1984; Petras, 1984), the design of the experiments would tend to obscure the paralysis issue. Fixed, sub-lethal doses were applied to small numbers of animals terminated at fixed intervals to pursue histological studies. In contrast, in the work reported here, large numbers of animals were involved, in many cases the absorbed dose was very close to lethal and the poisoning was allowed to run the course. It was among the near lethal responses that the paralysis was identified. Paralysis was not observed in any less challenged animals. The amount of OP absorbed was uncontrolled as it was the amount that could be/was absorbed in the window between contamination and decontamination. There were no attempts at medical intervention and no therapies were applied. Because brain damage has been identified with direct exposures to GD and GB without decontamination, it is very likely that the brain damage reported here was from the same cause, i.e. organophosphate poisoning and paralysis or some form of functional deficit would be logical results. However, because there was only minimal histology done, no causative response may be concluded from these studies. No attempts were made to define either behavioural or psychic damage in the subjects.

The paralysis observations described were incidental to the work involved for the development of the RSDL®. The recording of clinical signs immediate post exposure/decontamination was normally done only to track the toxic responses of the guinea pigs to establish the potency of the decontaminant. The paralysis was an unexpected side issue because the animal was recovering when these signs were manifest. The histology was an afterthought and so, unfortunately, only minimal sampling was done. Also because of the object of the work, there was much effort to ensure that the amount of agent applied and the formulation of the decontaminant were the only known uncontrolled variables. Therefore, these studies could not address the effects of either lower levels of exposure for longer times or variable amounts of skin surface contaminated which both translate roughly to the effects of variable rates of poisoning.

In the exposures to GD and VX described here, paralysis was observed only in animals that had been *in extremis*: either convulsions or flaccid paralysis. All had been prostrate from advanced poisoning. In some cases, the animals were thought to be dead and yet recovered (this was early in the experiments when the workers were less familiar with the toxicosis). As apnea and flaccid paralysis following tonic spasm were a regular occurrence, there was the potential for anoxic damage to the brain and spinal cord. However, previous studies (Petras, 1981, 1984) have indicated that anoxia may not be involved, albeit that different experimental conditions were involved. The CNS lesions on record apparently can be prevented by therapeutic intervention with anti-convulsant drugs (McLeod *et al.*, 1984; Sidell, 1997; Ballogh *et al.*, 1998; Wood and Tattersall, 1999) but no therapies were applied in the studies reported.

The only apparent relationship between the cutaneous doses of OP and the occurrence of any of the clinical signs or the paralysis was that the animals all reached an advanced clinical effect of the OP. No numerical values could be attributed because of the decontamination applied. Because the skin decontamination was generally effective and the work was approaching the optimal configuration for the decontaminant, the amounts of OP applied to the animals steadily increased. Eventually, the total amounts of OPs applied in the open to the backs

of the guinea pigs before decontamination were so large (>70 µl per animal; 26 animals at a time) that the work was stopped for safety reasons, the potency of the decontaminant having been well established.

The incidences of clinical signs recorded in Tables 1 and 2 are those actually observed and may not present the full picture for each animal. The experiments from which the data were derived were specifically designed to demonstrate the potency of the RSDL for decontaminating OPs on skin (Bide *et al.*, 1988a,b, 1989, 1992; Bide and Risk, 1991; Sawyer *et al.*, 1991) and were conducted over a period of several years. On a given day, the experiments usually involved a number of animals simultaneously and the experimenters were occupied with the contamination and decontamination procedures. Some animals went rapidly from convulsions to death while others were being decontaminated and washed. The result was that the occurrences and sequences of the clinical signs were not always recorded on time. Also, the convulsions were not always violent. As the animals were restrained, mild convulsions may have been overlooked. Further, some animals were in the early stages of toxicosis at the end of the working day and the progression of clinical signs occurred after hours. Certainly, a number of paralysed animals were discovered the morning following the exposures.

In human exposures to OPs, the casualties may range from very slight contacts to near fatal cases (Okumura *et al.*, 1996, 1998a,b). In cases of civilian exposures, pretreatments and immediate therapy are unlikely. In military operational exposures, it is probable that there will be cases with and without pretreatments, with a range of times from contamination until treatment was applied (if at all) and the extent of contamination will be unknown. Further, in cases where treatments have been applied, there will be little or no control over the timing of the interventions relative to the appearance of clinical signs, to time/duration of exposure or to the time from exposure or treatment or triage. Under these conditions, the immediate sequellae described in the guinea pigs in this study may be duplicated in human subjects. A delayed paralysis has been described in the human (Bidstrup and Bonnell, 1954). The paralysed patient represents a significant drain on medical facilities for both immediate and long term care. Therefore, until there is evidence to the contrary, paralysis should be added to the list of concerns to be considered when assessments are made of the medical requirements for treating, handling, recovery support and long term care of casualties from OP poisonings.

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DRDC Suffield Animal Care Statement

In conducting the research described in this report, the investigators adhered to the 'Guide to the Care and Use of Experimental Animals, Vol. I, 2nd edn' published by the Canadian Council on Animal Care.

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