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Towards catch-up therapy: evaluation of nucleophilic active pharmaceutical ingredients for the treatment of percutaneous VX poisoning, in-vial and in-vitro studies

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ABSTRACT

Dermal exposure to low volatility organophosphorus chemical warfare agents (OP CWA) poses a great risk to the exposed person. Due to their lipophilic nature, these compounds rapidly absorb into the skin, leading to the formation of a "dermal reservoir" from which they slowly enter the bloodstream causing prolonged intoxication. Traditionally, strategies to counter the toxicity of such substances consist of chemical decontamination/physical removal of the residual agent from the skin surface (preferably as soon as possible following the exposure) and administration of antidotes in the case of intoxication signs. Hence, these strategies are unable to counter a substantial amount of the agent, which accumulates inthe dermal reservoir. More than a decade ago, the concept of a "catch-up therapy" intended to neutralize the dermal reservoir was suggested. Herein, we describe examples of potential "catch-up therapy" lotions - vehicles designed to deliver small nucleophilic molecules into the skin and potentially decompose the remaining CWA before it reaches the blood stream. Eleven nucleophilic compounds, based on approved drugs, were initially screened. They were then tested in various binary solutions, for their detoxification efficacy and degradation ability towards lipophilic OP CWA models such as dibutylphosphofluoridate and *o*-nitro-phenyl diphenyl phosphate, as well as the nerve agent VX, by means of kinetic ^{31}P NMR and UV–Vis spectroscopy. Of these, the potassium and diethyl ammonium salts of acetohydroxamic acid (AHAK and AHA DEA) in (DMSO/H_2O 1:4) were found to be the most active nucleophiles, hydrolyzing VX in practical time scales ($t_{1/2}$ = 5.28 and 6.78 min, respectively). The vehicle solution DMSO/H_2O 1:4 promoted the penetration of substantial amounts of AHA K and AHA DEA through excised pig skin in *in-vitro* studies, suggesting that such formulations may serve as useful CWA nucleophilic scavengers for both on and within -skin detoxification. These findings may pave the way to a more efficacious treatment against low volatility OP CWA percutaneous poisoning.

1. Introduction

Organophosphorus (OP) chemical warfare agents (CWA) are a group of highly toxic substances which exert their activity by irreversibly inhibiting acetylcholine esterase (AChE), the enzyme that hydrolyses acetylcholine (ACh) in nerve synapses. Inhibition of AChE leads to ACh accumulation which results in a cholinergic crisis (Costanzi et al., 2018; Thiermann et al., 2016). Although banned by international treaties (i.e., Organization for the Prohibition of Chemical Weapons- OPCW), the deployment of OP CWA against civilians is well documented during the last decade (Syria 2013 and 2017, Kuala Lumpur, Malaysia 2017, Salisbury, UK 2018, etc.).

OP CWA largely vary in their physicochemical properties (e.g. volatility, solubility, chemical stability, etc.). This variability is expressed in their mode of exposure and toxicokinetics (Munro, 1994; Thiermann et al., 2016). Exposure to OP CWA may occur by different routes, primarily inhalation and percutaneous absorption. While volatile compounds are most likely to be absorbed mainly via the respiratory system, for low volatility compounds (e.g. the V-type nerve agents VX, VM, CVX and RVX), dermal uptake is expected to be a dominant route of entry (Joosen et al., 2017, 2010; Romano et al., 2008; Thiermann et al., 2016). The route of exposure in turn has a large impact on the toxicokinetics of poisoning. Accordingly, following exposure, volatile OP nerve agents are promptly absorbed through mucous membranes,

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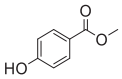
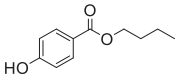
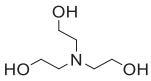
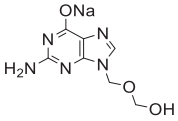
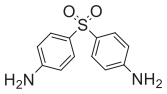
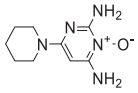
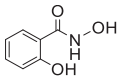
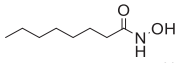
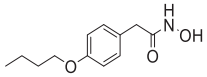
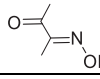
leading to fast (within minutes) onset of cholinergic symptoms. Conversely, low volatility lipophilic OP nerve agents absorbed into the skin, form a subcutaneous depot from which they distribute slowly into the body (Chilcott et al., 2005a, 2005b; Joosen et al., 2017, 2013, 2010). Thus, systemic signs of poisoning may appear only after a time lag which may take hours (Dalton et al., 2006; Joosen et al., 2010; Thiermann et al., 2016). Furthermore, high levels of the agent may persist in the blood circulation for hours (Joosen et al., 2010), due to continuous diffusion of the poison from the depot into the blood stream, combined with its chemical stability towards hydrolysis at physiological pH (Yang, 1999).

The aforementioned toxicological characteristics of low volatility nerve agents require special attention when devising appropriate decontamination and treatment strategies against them. Traditionally, strategies to counter the toxicity of nerve agents consist of two main tactics; decontamination of the contaminated skin surface, preferably by an active decontamination lotion such as RSDL® (Reactive Skin Decontamination Lotion) or AlldecontMED as soon as possible (these lotions were found to be effective if used within 15 min following exposure (Joosen et al., 2017; Thors et al., 2017a), and injection of antidotes in the case of intoxication signs (Hamilton et al., 2004; Thiermann et al., 2016). However, in a real-life scenario, toxicological signs following dermal exposure to persistent nerve agents, which will most likely be the trigger to treat, may be long delayed. Accordingly, by the time treatment will commence, a substantial amount of the agent will most likely already be absorbed into the skin, forming a dermal reservoir (Joosen et al., 2013; Wetherell et al., 2008). In such cases, late decontamination of the skin surface will probably be only partially effective (Chilcott et al., 2005a; Joosen et al., 2013; Wetherell et al., 2008). Furthermore, at this stage antidotal treatment will likely achieve only temporary relief, due to reoccurrence of symptoms following continuous penetration of VX from the dermal reservoir, necessitating repeated administration of antidotes (Bloch-Shilderman et al., 2019; Joosen et al., 2013, 2010). Thus, the aforementioned strategies are expected to be most effective only if the formation of the dermal depot is prevented (Chilcott et al., 2005a). For this purpose, new complementary strategies need to be considered.

Recently, Joosen and coworkers explored the efficacy of delayed decontamination by RSDL® (90 min following exposure) of hairless guinea pigs dermally exposed to VX, showing a delay in complete AChE inhibition. This effect was attributed to the partial neutralization of a VX depot within the skin following the decontamination, although a single decontamination was insufficient to prevent morbidity (Joosen et al., 2017). Similarly, Thors and coworkers showed a decrease in VX penetration rates through human skin following decontamination with RSDL®, even 120 min following exposure (Thors et al., 2017b). As it is highly likely that so long after exposure a substantial amount of the agent had already been absorbed to the skin and formed a depot (Chilcott et al., 2005a; Dalton et al., 2006), we may speculate that a small but sufficient portion of the RSDL® applied might have reached the dermal depot and neutralized some of the agent (although RSDL® was designed to neutralize the poison on the skin surface without penetration of its active ingredient (Connolly et al., 2020)). No explicit reference to such possibility was made.

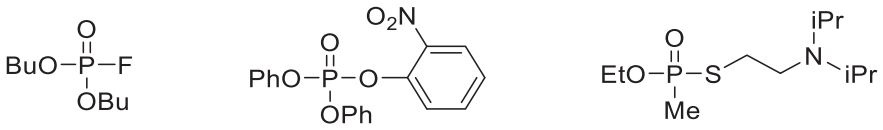
In line with the above, more than a decade ago, the concept of a "catch-up therapy" was suggested by Chilcott et al., who envisioned that reactive chemicals in an appropriate formulation could be delivered into the skin, follow the same pathway of the agent, and neutralize the dermal reservoir (Chilcott et al., 2005b, 2005a). However, to the best of our knowledge, to date this elegant concept remains at a theoretical level, with no such medical formulation currently available. Therefore, we aimed to develop potential "catch-up therapy" lotions intended to penetrate the skin, in order to chemically deactivate an existing CWA dermal reservoir before it reaches the blood stream. The possible benefit of such a lotion in comparison to currently available decontamination kits would be its dual mode of action: (i) Skin surface decontamination and (ii) Intra dermal CWA decomposition.

Table 1
Molecular structure, name and application of candidate compounds.

Compound number	Structure	Name	Application
1		Methyl paraben	Preservative, anti-fungal agent
2		Butyl paraben	Preservative, anti-bacterial/fungal agent
3		Triethanolamine	emulsifier
4		Aciclovir	Antiviral
5		Dapsone	Antibiotic
6		Minoxidil	Antihypertensive vasodialator
7		Salicylamide	Analgesic and antipyretic
8		Acetohydroxamic acid (AHA)	Antibiotic, urease inhibitor
9		Salicylhydroxamic acid (SHA)	Antibiotic, urease inhibitor
10		Caprylhydroxamic acid (CHA)	Preservative
11		Bufexamac (BUF)	Anti-inflammatory agent
12		2,3-Butanedione monoxime (DAM)	Active ingredient in the RSDL® lotion

Notably, OP CWA are all electrophilic by nature, consisting of a central P(V) atom. Accordingly, the above described "catch-up therapy" lotions should consist of nucleophilic molecules, able to chemically deactivate the OP CWA depot. However, delivering strong nucleophiles through the skin is far from trivial, as such charged molecules may be too hydrophilic to cross the lipophilic skin barrier (Boroujerdi, 1987). Thus, careful planning is required; first, in respect to the design of such a lotion, two primary parameters should be considered: a) the nucleophile of choice has to be active enough to efficiently neutralize the agent in a relevant time scale; b) the formulation has to enable the permeation of the charged nucleophile through skin layers. Obviously, these two requirements may contradict as the greater the nucleophilicity the higher the dipole moment and the hydrophilicity. Therefore, it can be assumed that a binary mixture of solvents will be the minimum needed to ensure both adequate solubility and reactivity of the scavenger, as well as its penetrability. Second, all components of the lotion should preferably be clinically approved for use in humans.

To address these demands, we scanned pharmaceutical libraries of approved drugs, in a search for active pharmaceutical ingredients that could be nucleophilic enough to neutralize OP CWA (**1**–**11**, Table 1), with an efficacy comparable to or higher than that of Diacetyl monoximate (DAM - the active ingredient of RSDL®, **12**). As lipophilic model OP's (G-type simulants) for initial screening, Dibutylphosphorofluoridate (**13**) and *o*-Nitro-phenyl diphenyl phosphate (**14**) were chosen, since their degradation rates could be measured by kinetic ^{31}P NMR



13 (DBPF) **14** (ONPDP) **15** (VX)

Fig. 1. Molecular structure of VX and G-type simulants DBPF and ONPDP.

and UV–Vis spectroscopy, respectively. The most active nucleophiles which degraded the OP models and nerve agent VX (**15**) at reasonable rates (Fig. 1), were evaluated for their skin penetration using flow-through diffusion cells in different formulations. To promote efficient permeation several techniques were applied, including formation of organic ion-pairs, use of permeation enhancers and use of concentrated solutions of the nucleophiles in a H_2O/DMSO binary solution. Herein we report our results, delineating new formulations that can penetrate the skin depot and hydrolyze VX at a reasonable timescale, which may accordingly be suitable for use as a "catch-up therapy" against cutaneous intoxication by OP's.

2. Materials and methods

Caution! *OP's are extremely toxic compound, with special emphasis on VX. Experiments with these compounds should only be performed by trained personnel using applicable safety procedures.*

2.1. Chemicals

The following active pharmaceutical ingredients, reagents and solvents (HPLC grade): methyl paraben, butyl paraben, triethanolamine, sodium aciclovir, dapsone, minoxidil, salicylamide, acetohydroxamic acid (AHA), salicylhydroxamic acid (SHA), caprylhydroxamic acid (CHA), Bufexamac (BUF), diacetyl monooxime (DAM), diethylamine (DEA), trimethylamine, ethanolamine, diethanolamine, piperidine, pyrrolidinoethanol (Epolamine), piperazine, dimethyl sulfoxide (DMSO), propylene glycol (PG), ethanol (EtOH), isopropanol (IPA), polyethylene glycol monoethyl ether (mPEG), sodium metoxide and potassium methoxide were purchased from commercial suppliers and used without further purification. Reactive Skin Decontamination Lotion (RSDL®) was obtained from Emergent Protective Products USA Inc. Deionized water was obtained from a laboratory water purification system.

2.1.1. Dibutyl-phosphorofuloridate (13)

To a solution of tributylphosphite (4.62 gr, 18.47 mmol, 1.0 equiv) in THF (50 mL) bromine (2.95 gr. 18.47 mmol, 1 equiv) was added dropwise at −78 °C until brown color persisted and the mixture was left to stir for 1 h. Thereafter, the mixture was warmed to −40 °C and cesium fluoride (8.43 gr, 55.5 mmol, 3 equiv) was added. The reaction was left to reach rt and stirred for 8 h. After reaction completion the solvent was evaporated under reduced pressure and the product was distilled (3.9 gr, 99.5% yield). Spectral data was identical to previously reported (Norlin et al., 2005).

2.1.2. 2-nitrophenyl diphenylphosphate (14)

To a solution of diphenylphosphinic chloride (4.83 gr, 2.04 mmol, 1.0 equiv) in ether (50 mL) triethylamine (2.06 gr, 2.04 mmol, 1.0 equiv) and 2-nitrophenol (2.84 gr, 2.04 mmol, 1.0 equiv) were added dropwise and the mixture was left to stir for 18 h. After reaction completion the organic phase was washed twice with water (50 mL ×2), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (Hexane to Hexane/EtOAc 1:5) to give **14** (6.26 gr, 95% yield). Spectral data was identical to previously reported (Jones et al., 2016).

2.1.3. O-ethyl-S-2-(N, N-diisopropylaminoethyl)methylphosphonothioate (VX, 15)

Was synthesized in house (>95% purity).

2.2. NMR spectroscopy

^{31}P {^{1}H} spectra were obtained at 202 MHz at room temperature on an 11.7 T (500 MHz) Bruker spectrometer (Avance III HD). Chemical shifts were calibrated to trimethyl phosphate as 0 ppm. The spectra were recorded using standard parameters of the TopSpin software (version 3.5). Each data point was obtained from 32 scans with a spectral width of 200 ppm and 4 s recycle delay. All spectra were taken under identical conditions and integration was done automatically using TopSpin 3.5.2 software and the build in AU multi_integ3.

2.3. Degradation kinetics

All nucleophiles, accept **3**, **5** and **6** which were used as free base, were converted to sodium, potassium or ammonium salts prior to kinetic evaluation by reacting them with sodium methoxide, potassium methoxide or the appropriate amine, respectively. Nucleophiles (26 equiv) were added to DMSO or PG/H_2O 1:1 solution (1 mL) and stirred for 1 min until completely dissolved. DBPF (**13**) or VX (**15**) (2 μL, 1 equiv) were carefully added, and the solution was transferred to an NMR tube for kinetic analysis. Kinetic NMR acquisition started as soon as possible (without lock and shimming) and continued until full degradation, or until no further change in reaction conversion was observed. Analysis of the results was performed by the GraphPad Prism 5 software to determine half-life times.

2.4. UV–Vis spectroscopy

For kinetic experiments a stock solution of *o*-nitro-phenyl diphenyl phosphate (ONPDP) was prepared by dissolving 5 μL (18.85 μmol, 1 equiv) in 1 mL of DMSO. Accordingly, stock solutions for selected nucleophiles were prepared in ethanol with 1:10 ONPDP: nucleophile ratio (188.53 μmol, 10 equiv). For a typical reaction, 36 μL of ONPDP and the appropriate nucleophile stock solutions were added to a cuvette containing 2928 μL of buffer/solvent 1:1 solution. The buffer (pH 9.2) was prepared by mixing 10% (v/v) Na_2CO_3 (0.1 N) with 90% (v/v) $NaHCO_3$ (0.1 N) solutions. Kinetic measurements were carried out on a Shimadzu UV-1800 spectrophotometer using a Kinetics built-in function. Analysis of the results was performed using the GraphPad Prism 5 software to determine half-life times.

2.5. pK_a measurements

The pK_a values of hydroxamic acids and DAM were determined pH metrically using an Eutech Instruments (PH 700) pH meter. The instrument was calibrated with pH 4, 7 and 10 standard buffer solutions (Reagecon).

2.6. Log P measurements

Measurements of the partition coefficient were performed with *n*-octanol as the oil phase and water as the aqueous phase, in accordance

with the OECD guidelines (OECD/OCDE, 1995). The two phases were mixed for 24 h to form saturated phases. Solutions of AHA (**8**) and DAM (**12**) (1 mM) were prepared in the aqueous phase and solutions of salts were prepared in the aqueous phase adjusted to pH 11. The aqueous phases were mixed with *n*-octanol in 10 mL tube at the following portions:

(1) Water/*n*-octanol 1:1 (5 mL each)
(2) Water/*n*-octanol 1:2 (aqueous phase 3 mL *n*-octanol phase 6 mL)
(3) Water/*n*-octanol 2:1 (aqueous phase 6 mL *n*-octanol phase 3 mL)

Duplicates of each solution in tubes were closed with a cap and shaken for 24 h at room temperature. After careful separation using centrifugation and by a syringe, the concentration at each phase was evaluated by means of HPLC. The partition coefficient was calculated as the ratio between the concentrations of the two phases. Three solutions with different ratios of water/*n*-octanol were used to ensure that the results are not erroneous due to saturation of one of the phases with the tested compounds.

2.7. In vitro skin penetration

Portions of (5 × 5 cm) of excised domestic pig back skin free of fat (obtained from Lahav CRO, Israel) were carefully cleaned and stored at −80 °C. Prior to use the skin portions were thawed at 4 °C overnight. Flow-through diffusion cells were used throughout the study. PBS was chosen as the receptor fluid since it is commonly used in similar studies and as it follows the OECD guidelines which state that a test material should be soluble up to ten times the likely maximum concentration achievable in the receptor fluid during the experiment (Pendlington, 2008). The solubility of the various salts of **8** and **12** was found to be orders of magnitude higher than that required by the above guidelines. During the experiments, the solution in the receptor chamber was maintained at 32 °C (using a HAAKE P5 circulating water bath) and stirred with Teflon-coated magnetic stirring bars. The skin was mounted between the donor and receptor compartments with a pinch clamp with the stratum corneum facing the donor compartment and the dermis facing the receptor compartment (exposed area 3.46 cm^2). All receptor cells were filed with PBS (pH 7.4) and connected to peristaltic pumps (Ismatec 2/12). The receptor solution was continuously pumped at a flow rate of 166 μL/min. Donor compartments were covered with plastic lids and the system was equilibrated for 20 min. After equilibration 2 mL of the formulation was added to the donor compartments and the solution was covered with a plastic lid. Receptor solution was continuously collected into 5 mL tubes by an automatic fraction collector (ISCO RETRIEVER IV) for 30 min intervals, up to 8 h, and immediately sealed. The formulation in the donor compartment was left on the skin for 30 min and then removed by washing four times with PBS solution.

2.8. Sample analysis

The receptor samples from permeation experiments, collected over 0.5 h intervals, were analyzed using high-performance liquid chromatography (Dionex Ultimate 3000). Aliquots of 10 μL or 40 μL from the samples were injected into the column (Phenomenex, Gemini 5 μm C18 110 Å). The mobile phase consisting of a mixture containing acetonitrile and water (1:99 and 50:50, v/v, for AHA and DAM respectively), adjusted to pH 2 by phosphoric acid, was used as the eluent. The flow rate was set to 0.7 mL/min and the column was kept at 35 °C. Detection was performed at 210 nm, using a UV detector.

2.9. Data treatment and statistical analysis

Quantification of the nucleophiles was done using calibration curves, which were generated for each salt by injecting known standard solutions of the salts in PBS. The slopes were calculated from all the curves that covered a linear range of 0.5–120 μg/ml, $r^2 > 0.998$. Permeation was evaluated by plotting the cumulative amount of permeate passing per cm^2 of skin versus time. In cases in which steady state permeation was reached, the absorption rates (J_{ss}) were determined as the slopes of the linear portion of the plot. The lag times (T_L) were calculated from linear extrapolation of the steady state portion of the plot. The permeability coefficients (K_p) were calculated as the ratio between the absorption rates and the initial concentrations of the permeates in the applied formulations. Total accumulations (Q_8) were calculated as the cumulative amount of permeate after 8 h. Each experiment was evaluated by four independent replicates. The integrity of the skins was assessed by examining the permeation results. Skins that were damaged and exhibited excessive penetration rate (>10,000 μg $cm^{-2}h^{-1}$ without reaching steady state) were removed from the analysis and replaced by additional experiments. All skin permeation results are presented as the mean ± standard error of the mean (SEM). Statistical analysis was performed using a one-way ANOVA followed by either a Dunnett's or Bonferroni multiple comparison test to determine differences in penetration rates. Results were considered significant for $P < 0.05$.

3. Results and discussion

3.1. Initial screening of potential nucleophiles

The preliminary step of this work was to identify and evaluate potential compounds, from pharmaceutical origin, for subcutaneous neutralization of OP CWAs. Thus, we sought after Active Pharmaceutical Ingredients (APIs) and excipients in the pharmaceutical and cosmetics industries, that may fulfill the following criteria. 1) Such APIs must be nucleophilic in order to react with the electrophilic OP's. They must also bear a functional group which is deprotonated at pH < 12 in order to avoid skin damage. Such compounds may be hydroxyls (with emphasis on phenols which have a lower p*K*a than aliphatic alcohols), carboxylates, amines, oximes and hydroxamates as their conjugated base would serve as a strong nucleophile. 2) The APIs should be of the lowest molecular weight possible, as chemicals with a molecular weight greater than ~500 Da do not penetrate the skin efficiently (the 'rule of 500') (Bos and Meinardi, 2000). 3) The APIs should be the least toxic possible. 4) Simple structured and non-expensive compounds are preferred. Based on these criteria, we scanned several pharmaceutical databases: Drug Bank web site (Wishart et al., 2018), Orange Book– FDA web site ("U.S. Food and Drug Administration Orange Book.,") and Handbook of Pharmaceutical Excipients (Rowe et al., 2013). Consequently, we located a handful of APIs that meet the threshold requirements 1–3 and may be used as potential nucleophiles for this purpose. Among those are phenols (**1**, **2**, **4**, **7**), amines (**3, 5, 6**) and hydroxamic acids (**8**–**11**) (Table 1).

Beyond the nucleophiles, additional key aspects also need to be considered in the formulation design: (i) VX (**15**) has low solubility in aqueous solutions (Yang et al., 1992). Therefore, the formulation has to be based (at least partially) on an organic solvent that could solubilize VX (**15**) in order to facilitate its hydrolysis. (ii) It is desirable that the solvent would accelerate nucleophilic substitution reactions. This should be achieved either by stabilizing the transition state or destabilizing the ground state, as was previously established mechanistically (Ashkenazi et al., 2010; Hamlin et al., 2018). (iii) It is advisable that the solvent would serve as a permeation enhancer in order to increase the permeation of the nucleophile through the stratum corneum (Williams and Barry, 2004). (iv) The formulation should preferably solubilize high quantities of the nucleophile, to assure sufficient hydrolytic and permeation rates.

Keeping in mind the high toxicity of VX which requires special safety measures, and the fact that 11 candidate APIs multiplied by 3–4 potential cations (both organic and inorganic), all multiplied by a few possible vehicles, would form a matrix of ca. dozens of kinetic experiments, we decided to reduce the list of possible API's by initially examining their nucleophilic capabilities towards model OP's. These

Table 2
Kinetic rates ($t_{1/2}$) of **13** hydrolysis by compounds **1**–**12**.*

Compound number	Compound Name	Salt form	Solvent	$t_{1/2}$ (min)
1	Methyl paraben	Na	DMSO	2.64
2	Butyl paraben	Na	DMSO	2.94
3	Triethanolamine	Free base[a]	DMSO	N.R.
4	Aciclovir	Na	DMSO	102.48
5	Dapsone	Free base[a]	DMSO	N.R.
6	Minoxidil	Free base[a]	DMSO	N.R.
7	Salicylamide	Na	DMSO	N.R.
8	Acetohydroxamate (AHA)	Na	DMSO	<1
9	Salicyl hydroxamate (SHA)	2 × Na[b]	DMSO	<1
10	Caprylhydroxamate (CHA)	Na	PG/H_2O 1:1	<1
11	Bufexamac (BUF)	Na	PG/H_2O 1:1	<1
12	DAM	Na	DMSO	<1

* In the absence of a nucleophile no observable decomposition in DMSO or PG/H_2O 1:1 at rt could be detected by ^{31}P NMR, during the relevant time frame (60 min.).
[a] Amines were used as free base.
[b] Salicyl hydroxamate (SHA) used as di-sodium salt. N.R-no reaction detected.

OP's (**13** and **14**) were chosen based on the fact that they are also sparingly soluble in water (resembling VX) but possess a better leaving group than VX (F and *o*-nitrophenol in comparison to di-*i*-propylaminoethyl thiol). Thus, those nucleophiles who fail to cleave the model compounds within sufficient time scale, would certainly be inadequate for neutralizing VX. First, the selected nucleophiles were reacted with **13**, a lipophilic G-type simulant, in DMSO (100 mM) (except for **10**–**11**, which were insoluble in DMSO and were therefore tested in PG/H_2O 1:1 solution) and followed by ^{31}P NMR spectroscopy. Diacetyl monooximate (DAM**, 12**), the active ingredient of RSDL® , was used as a reference, since it is approved for skin decontamination and has been widely tested as a scavenger (Elsinghorst et al., 2015; Schwartz et al., 2012; Thors et al., 2017b). Half-life times ($t_{1/2}$) were calculated from the kinetic data and are summarized in Table 2. Among the tested compounds, hydroxamates (**8**–**11**) were the most active, rapidly degrading DBPF with $t_{1/2} < 1$ min (due to the intrinsic NMR time scale limitations, more accurate reaction rates for **8**–**11** cannot be determined). Phenols were slightly less active, degrading **13** with a half-life of 2.64 min and 2.94 min for compounds **1** and **2**, respectively. The rest of the tested compounds showed very low activity, $t_{1/2} = 102.48$ min for sodium aciclovir (**4**) or none with amines **3** and **5**. As these rates are impractical for our purposes, especially while taking into account that phosphonothiolates are less prone to hydrolysis than phosphofluoridates (Marciano et al., 2012; Yang et al., 1992), we excluded compounds **1**–**7** from any further study. These results are in accordance with previous studies examining hydroxamate ions as potential nucleophiles towards electrophilic centers such as organophosphorus nerve agents (Medeiros et al., 2012; Mello et al., 2011; Orth et al., 2011, 2009; Satnami et al., 2010; Silva et al., 2009; Swidler and Steinberg, 1956; Wong et al., 2019).

3.2. Reaction condition optimization and solvent selection

In order to optimize reaction conditions and differentiate between reaction rates, we synthesized another simulant, o-nitro-phenyl diphenyl phosphate (ONPDPP, **14**), which carries a nitrophenol group and can be followed by UV spectroscopy. Hydrolytic activity of the hydroxamates was tested in 0.2 mM solutions containing carbonate buffer (pH 11) with five different solvents commonly used for topical formulations (DMSO, PG, EtOH, IPA, and mPEG), at a 1:1 ratio

Table 3
Kinetic rates for the hydrolysis of **14** by sodium hydroxamates **8**–**11** and **12** (DAM) in different solvents.

Compound Number	Name[a] Name		$t_{1/2}$ (min)/Solvents[b]			
	AHA	DMSO	PG	EtOH	IPA	mPEG
8	SHA	2.6	16.8	31.1	48.9	10.5
9	CHA	13.2	17.2	41.5	66.1	N.A.
10	BUF	3.9	6.8	17.5	29.2	7.7
11	DAM	4.3	10.3	18.2	26.8	N.A.
12		9.0	21.3	55.9	55.6	N.A.

[a] All nucleophiles were used as sodium salts.
[b] All reactions were performed in a mixture of solvent/buffer 1:1.

Table 4
p*K*a values of lead nucleophiles at DMSO/H_2O mixtures.

% DMSO (v/v)*	pKa AHA (**8**)	CHA (**10**)	BUF (**11**)	DAM (**12**)
0	9.55	9.6	9.3	9.6
20	10	10.3	10.2	10
50	11.4	11.7	11.4	11.4
80	13.3	13.7	13.4	13.6

* All pKa measurements performed in DMSO/H_2O mixtures.

(Table 3). Among the tested solutions Buffer/DMSO 1:1 gave the highest reaction rates and **8** was the most active nucleophile in this solution hydrolyzing **14** with a half-life time of 2.6 min. In protic solvents (PG, EtOH and IPA) the most active nucleophiles were **10** and **11**, although with rates lower than in DMSO. In all tested cases **9** and **12** were the least active nucleophiles. Notably, in the absence of nucleophile (**8**–**12**) only a minor decomposition of **14** could be observed at rt, with rates slower by 15–60 fold. This contribution to the decomposition, which can be attributed to the basic pH of the buffer, is negligible during the relevant time frame (minutes). As **9** was clearly found to be the inferior among all other hydroxamic acids, we omitted it from the following studies.

3.3. pK_a measurements

Since the concentration of the deprotonated hydroxamate ion

Table 5
Log P and kinetic rates of VX hydrolysis by salts of AHA and DAM.

Entry	Nucleophile	Salt form	Conditions	LogP	$t_{1/2}$ (min)
1	DAM (**12**)	K (**12a**)	mPEG/H_2O 9:1	−0.635	5.71
2	AHA (**8**)*	K (**8a**)	mPEG/H_2O 9:1	−2.17	7.45
3	AHA (**8**)	K (**8a**)	DMSO/H_2O 1:1	−2.17	5.14
4	AHA (**8**)	K (**8a**)	DMSO/H_2O 1:4	−2.17	5.28
5	AHA (**8**)	Na (**8b**)	DMSO/H_2O 1:4	−2.42	8.91
6	AHA (**8**)	Diethylamine (**8c**)	DMSO/H_2O 1:4	−2.06	6.78
7	AHA (**8**)	Diethylamine (**8c**)	DMSO/H_2O 1:1	−2.06	16.89
8	AHA (**8**)	Triethylamine (**8d**)	DMSO/H_2O 1:1	–	30.51
9	AHA (**8**)	Ethanolamine (**8e**)	DMSO/H_2O 1:1	–	37.65
10	AHA (**8**)	Diethanolamine (**8f**)	DMSO/H_2O 1:1	–	42.92
11	AHA (**8**)	Epolamine (**8g**)	DMSO/H_2O 1:1	–	33.86
12	AHA (**8**)	Piperazine (**8h**)	DMSO/H_2O 1:1	–	31.49

* Log P of AHA acidic form is −1.58.

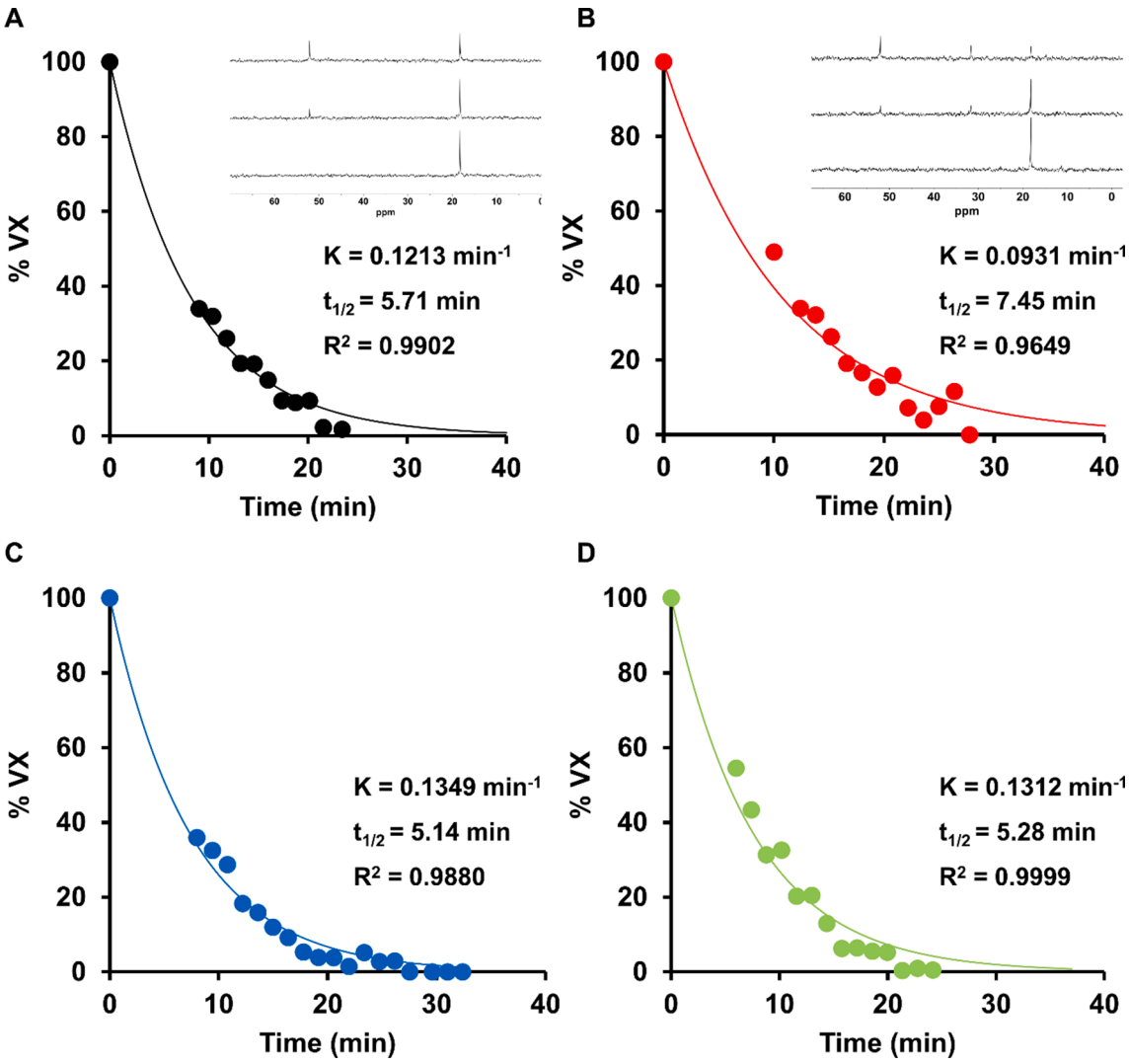


Fig. 2. VX decomposition rates and rate constants (min^{-1}) using: A) **12a** in mPEG/H_2O 9:1; B) **8a** in mPEG/H_2O 9:1; C) **8a** in DMSO/ H_2O 1:1; D) **8a** in DMSO/ H_2O 1:4 measured by ^{31}P NMR spectroscopy. Inset, ^{31}P NMR spectra of VX degradation by: A) **12a** in mPEG/H_2O 9:1; B) **8a** in mPEG/H_2O 9:1. The Low field signal corresponds to VX and the high field signal corresponds to EMPA.

directly influences the rate of hydrolysis, the pK_a values of nucleophiles **8**, **10**–**12** were determined pH-metrically in three H_2O/DMSO mixtures (20%, 50%, 80% DMSO) in order to find the lowest pH sufficient for full ionization of the hydroxamates (Table 4). For all hydroxamic acids the pK_a increased with the increase in DMSO fraction and it was in proximity to 10 in 20% DMSO (v/v) and 11.4 in 50% DMSO (v/v). Therefore, the ionization percent in formulation with pH 12 in 20% DMSO mixture will be 99% while in 50% DMSO mixture only 80% will be ionized.

Attempts to dissolve **10** and **11** or their sodium and potassium salts in H_2O/DMSO or H_2O/PG mixtures, in high concentrations (>50 mg/mL), failed. DMSO is a polar aprotic solvent known to accelerate nucleophilic reactions, especially those involving α-nucleophiles (Ghosh et al., 2005). In addition, DMSO is used as a permeation enhancer and approved for human use (Williams and Barry, 2004). Therefore, due to the low solubility of CHA and BUF salts, we decided to exclude them from any further study and continue exploring only DMSO based formulations of AHA salts.

3.4. Log $K_{O/W}$ measurements

Partition coefficient values of **12**, **8** and several of their salts were measured. The potassium salt of DAM was the least negative (logP = −0.635). The salt forms of AHA (**8a-c**) have lower logP values in comparison to the free acid **8** (logP = −1.58, Table 5), as expected. Therefore, such increased hydrophilicity of the salts may hinder the permeation through the lipophilic skin. Nevertheless, it was previously shown that high solubility, formation of organic ion-pairs and the use of permeation enhancers can compensate for the low permeation due to high hydrophilicity (Boroujerdi, 1987; Serajuddin, 2007; Williams and Barry, 2004). Such ideas were successfully implemented in many commercial formulations including diclofenac, a non-steroidal anti-inflammatory drug, e.g. Pennsaid® (45.5% DMSO formulation) and Voltaren emugel® (diethyl amine salt) (Fini et al., 2012, 1999; Fuller and Roth, 2011). In order to test this concept, we prepared a variety of AHA ion-pairs with aliphatic amines as counter ions (Table 5, Entries 7–13). The diethyl ammonium salt showed slightly higher affinity to the lipophilic phase (logP = -2.06) than the inorganic salts of AHA, providing some estimate to the relative permeation of the salts through the skin.

3.5. VX degradation efficiency

Based on the kinetic experiments and the pK_a measurements, H_2O/DMSO solutions (0.2 M) were chosen as the solvent for VX degradation experiments. The degradation of VX by salts of AHA (**8a-h**) and DAM (**12a**) in H_2O/DMSO and mPEG/H_2O (RSDL® vehicle) solutions was monitored using ^{31}P NMR spectroscopy. Notably, VX remains intact for

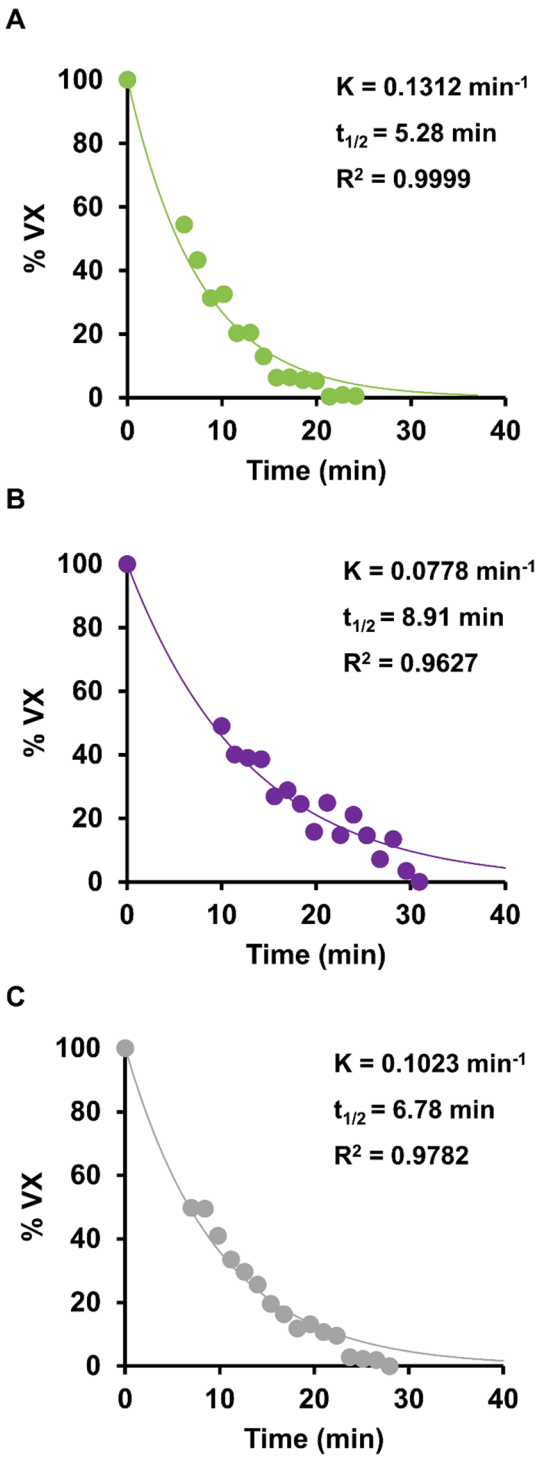


Fig. 3. VX decomposition rates and rate constants (min^{-1}) using: A) **8a** in DMSO/ H_2O 1:4; B) **8b** in DMSO/ H_2O 1:4; C) **8c** in DMSO/ H_2O 1:4 measured by ^{31}P NMR spectroscopy.

many hours while dissolved in these solutions, which have a neutral pH in the absence of the nucleophile, as may be anticipated based on to the literature (Yang, 1999). Consequently, the first kinetic data point of t = 0 min. can be set to 100% VX. All reaction profiles exhibited pseudo-first order kinetics (Figs. 2–4, S1–S30 in the SI). Following the reaction of **12a** with VX the only and final degradation product was ethyl methylphosphonic acid (EMPA) (Fig. 2**a**). Conversely, with **8** as the nucleophile, an acetamidophosphonate intermediate was observed as well, suggesting that the Lossen rearrangement step of the intermediate to form EMPA is slower than the initial step of **8** under these conditions (Fig. 2**b**) (Bierwisch et al., 2016). The degradation of VX by the potassium salt **8a** was as efficient as **12a** under all conditions ($t_{1/2}$ = 5.14–7.45 min, Table 5, entries 1–4 and Fig. 2, A-B), while the sodium salt **8b** was slightly slower ($t_{1/2}$ = 8.91 min, Table 5, entry 5 and Fig. 3, B). Organic salts of AHA (**8c-h**) in DMSO/H_2O 1:1 showed slower rates in comparison to the inorganic salts (Table 5, entries 7–12 and Fig. 4). The diethylamine salt **8c** was the most active among the tested organic salts ($t_{1/2}$ = 16.89 min). Interestingly, lowering the amount of DMSO in the vehicle mixture increased the hydrolysis rate (Table 5, entries 6–7). This influence can be explained by the fact that decreasing the relative volume of DMSO in the solvent mixture increases the dielectric constant of the solution, lowering the pK_a value of **8** (see Table 4) and thus enhancing the formation of the reactive 'ion pair' between the hydroxamate and the ammonium cation (Ghosh et al., 2005). This phenomenon is obviously less pronounced for inorganic cations (Table 5, entries 3–4) as the potassium is practically insoluble in DMSO. Nonetheless, it is clear that reasonable hydrolysis rates of VX can be obtained with either inorganic or DEA salts of AHA (Larsson et al., 2021).

Based on the above described kinetic results, together with the measured partition coefficients (Tables 2, 3 and 5 and Figs. 2–4) and the pK_a values (Table 4), it became clear that among all the nucleophilic API's and solutions vehicles we scanned, the most promising candidates, at least according to their chemical properties, would be AHA salts (**8a** and **8c**) dissolved in DMSO/H_2O 1:4. Therefore, we proceeded to the next step of measuring the skin permeation with only a handful of formulations, consist of **8a** or **8c** as the active nucleophiles.

3.6. Skin permeation

The effect of solvent, counter ion and concentration on the penetration of **8** through excised pig skin was studied with three different solutions (mPEG/H_2O 9:1, H_2O/DMSO 4:1, H_2O) in comparison to RSDL®, using flow-through diffusion cells. Solutions of **8** or RSDL® (2 mL) were applied onto the pig skin for 30 min and then removed by washing the skin four times with PBS solution (4 ×2 mL). Receptor samples were collected each 30 min (8 h experiment) and analyzed by HPLC. Steady-state absorption rates (J_{ss}), 30 min accumulation ($Q_{0.5}$), total accumulation (Q_8) and the lag time (T_L) were calculated from the results. Due to differences in the solubility of AHA salts in the tested solutions and hence the concentrations, permeability coefficients (K_p) which are the steady state Fluxes (J_{ss}) normalized by concentration, were calculated and are summarized in Table 6. Steady-states were achieved in all cases after lag times spanning from 2.35 to 3.78 h (Fig. 5). Steady-state absorption rates (J_{ss}) for all solutions applied, except for **8a** in mPEG/H_2O 9:1, were significantly larger than those for RSDL®. The penetration of **8a** in water and H_2O/DMSO 4:1 was significantly larger than that in mPEG/H_2O 9:1 ($P < 0.05$). The results also show that in comparison to water, 20% DMSO exhibited a marginally positive effect on skin permeation rates of **8a**, with an enhancement ratio of 1.13, while 90% mPEG exerted an inhibiting effect. Thus, it may be concluded that AHA salts penetrate the skin better than DAM salts, especially when dissolved in a highly polar media (i.e·H_2O/DMSO mixture). This, coupled with the fact that salts of **8** are by far less toxic than **12** (Ellin and Henry Wills, 1964; Griffith and Musher, 1975), emphasizes the skin scavenging potential of the former. We next tested the effect of the concentration of **8a** on its penetration. Three different solutions in H_2O/DMSO 4:1 were studied (217.2 mg/mL, 539.4 mg/mL and 880.9 mg/mL - Table 6, entries 4–6, respectively). The most concentrated solution showed the highest rate of absorption and total accumulation (Table 6, entry 6 and Fig. 5**A-B**, blue lines). However, no linear correlation between concentration and rate of absorption/total accumulation was observed. The more lipophilic **8c** was also tested in two concentrations (348 mg/mL and 548 mg/mL - Table 6, entries 7–8, respectively). The absorption rate of the concentrated solution was significantly higher than that of the less concentrated one. Moreover, this solution of **8c**

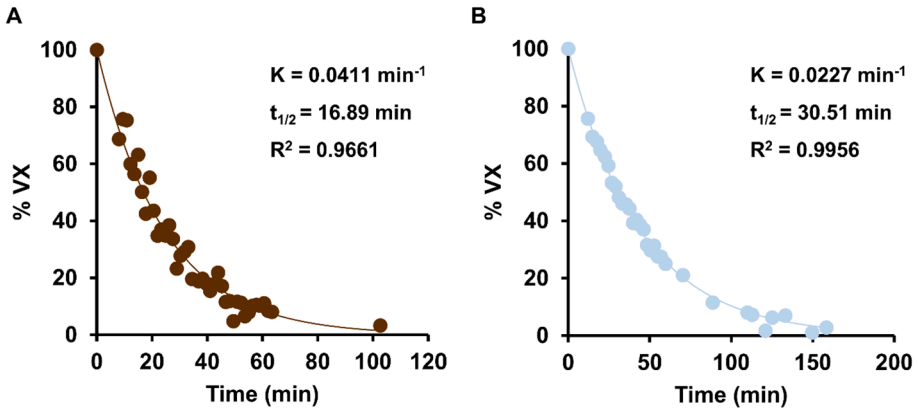


Fig. 4. VX decomposition rates and rate constants (min^{-1}) using: A) **8c** in DMSO/ H_2O 1:1; B) **8d** in DMSO/ H_2O 1:1 measured by ^{31}P NMR spectroscopy.

Table 6
The permeation parameters of **8** salts and RSDL® in different formulations.

Entry	Nucleophile	Vehicle	J_{ss} (μg $cm^{-2}h^{-1}$)	TL (h)	$Q_{0.5}$ (μg cm^{-2})	Q_8 (μg cm^{-2})	Co (mg mL^{-1})	Kp (10^6 cm h^{-1})
1	**12a** [a]	mPEG/H_2O 9:1	49.59 ± 6.77	3.3 ± 0.07	0	234.15 ± 34.7	175.39	282.72 ± 38.62
2	**8a**	mPEG/H_2O 9:1	122.52 ± 9.10	2.35 ± 0.27**	2.89 ± 0.36***	695.33 ± 85.41	115.74[b]	1058.62 ± 78.59*
3	**8a**	H_2O	375.05 ± 25.40*	3.78 ± 0.09	0.31 ± 0.15	1581.11 ± 95.21	205.75	1822.78 ± 123.47***
4	**8a**	H_2O/DMSO 4:1	423.39 ± 52.73**	3.27 ± 0.21	1.24 ± 0.23	2036.42 ± 327.54*	217.24	1949.17 ± 242.73***
5	**8a**	H_2O/DMSO 4:1	350.32 ± 80.93*	3.19 ± 0.10	0.38 ± 0.14	1679.32 ± 371.33	538.39	649.49 ± 150.04
6	**8a**	H_2O/DMSO 4:1	621.65 ± 39.45***	2.87 ± 0.05	1.15 ± 0.23	3168.70 ± 199.73***	880.92	705.68 ± 44.78
7	**8c**	H_2O/DMSO 4:1	448.20 ± 87.61**	3.25 ± 0.31	2.58 ± 0.83***	2212.41 ± 510.67*	347.81	1288.63 ± 251.89**
8	**8c**	H_2O/DMSO 4:1	1181.83 ± 126.79***	2.73 ± 0.21	1.62 ± 0.39*	6253.15 ± 883.93***	547.73	2157.69 ± 231.48***

Abbreviations: J_{ss}, Steady state flux; T_L, lag time; $Q_{0.5}$, amount permeated after 0.5 h; Q_8, amount permeated after 8 h; C_o, initial concentration; K_p, permeation coefficient. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to the corresponding value of RSDL®. Values are presented as mean ± SEM (n = 4).

[a] RSDL® contains in addition to DAM K (Dekon 139) 5% of the DAM acidic form.

[b] Due to low solubility maximal concentration of AHA K in mPEG/H2O 9:1 were used and not 200 mg mL-1.

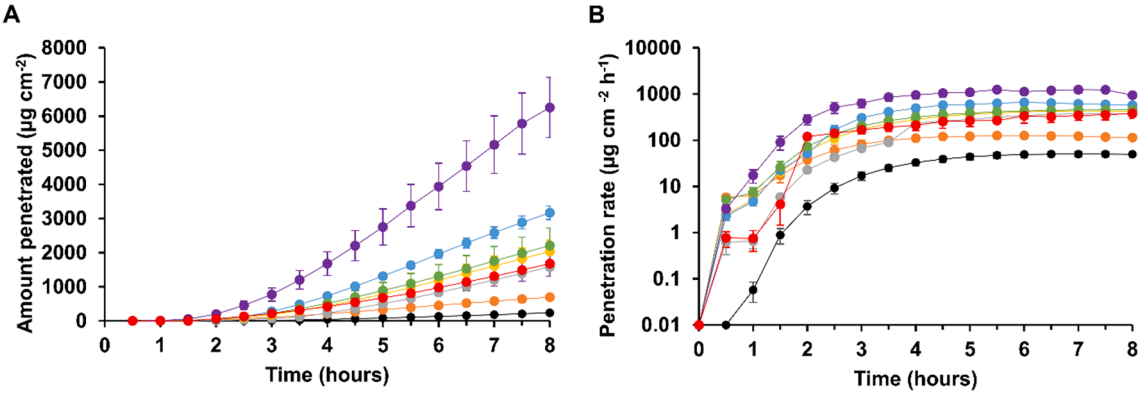


Fig. 5. Penetration profile of **8** in different formulations through excised pig skin in comparison to RSDL®. A) Amount penetrated and B) penetration rate following application of different formulations for 30 min; RSDL® (black), **8a** in mPEG/H_2O 9:1 (orange), **8a** in H_2O (gray), **8a** (217 mg/mL) in H_2O/DMSO 4:1(yellow), **8a** (538 mg/mL) in H_2O/DMSO 4:1(red), **8a** (881 mg/mL) in H_2O/DMSO 4:1(blue), **8c** (348 mg/mL) in H_2O/DMSO 4:1 (green), **8c** (548 mg/mL) in H_2O/DMSO 4:1 (purple). Values are presented as mean ± SEM (n = 4).

showed the highest rate of absorption (J_{ss} = 1181.83 ± 126.79 μg $cm^{-2}h^{-1}$), total accumulation (Q_8 = 6253.15 ± 883.93 μg cm^{-2}) and permeation coefficient (Kp = 2157.69 ± 231.48 cm $h^{-1} \times 10^{-6}$) among all tested formulations (Table 6, entries 1–8 and Fig. 5**A-B**, purple lines). It is noteworthy that the lag time (T_L, approx. 3 hr) is fairly consistent regardless of the different formulations. However, a delayed treatment might require that a substantial amount of scavenger would penetrate the skin as promptly as possible. In this regard, using a highly concentrated and more lipophilic formula may accelerate the nucleophile's diffusion and induce VX hydrolysis even before steady-state absorption is reached (e.g.; high concentration of **8c** in H_2O/DMSO 4:1).

Permeation results demonstrated that **12a** can, to some extent, permeate from the RSDL® lotion (mPEG/H_2O 9:1 solution). However, the permeation rate that we measured (49.59 ± 6.77 μg $cm^{-2}h^{-1}$) is substantially lower than that expected for efficient VX skin depot neutralization. Indeed, it is possible that RSDL® was designed this way in order to prevent the permeation of its toxic ingredients, while sustaining good hydrolytic activity for external skin decontamination (Connolly et al., 2020; Wong et al., 2020). Previously, Vallet and coworkers showed that VX absorption rates through pig and human skin under finite conditions are 90.5–129 μg $cm^{-2}h^{-1}$ and 45.5–57.5 μg $cm^{-2}h^{-1}$, respectively (Vallet et al., 2008). A similar study conducted by Dalton et. al showed even higher rates of pig and human skin permeation, albeit under infinite VX dose application conditions (207 ± 62 and

Table 7
Amount of AHA salts absorbed into the skin after 30 min.

Entry	Nucleophile	Vehicle	Co (mg mL^{-1})	$A_{0.5}$ [a] (mg cm^{-2})	% of applied dose
1	**12a**	mPEG/ H_2O 9:1	175.39	3.51 ± 1.92	3.46 ± 1.90
2	**8a**	mPEG/ H_2O 9:1	115.74	25.02 ± 4.99	37.40 ± 7.46
8	**8a**	H_2O	205.75	37.63 ± 10.53**	31.64 ± 8.85
3	**8a**	H_2O/ DMSO 4:1	217.24	20.25 ± 14.65	4.49 ± 0.92
4	**8a**	H_2O/ DMSO 4:1	538.39	15.99 ± 2.76	5.13 ± 0.89
5	**8a**	H_2O/ DMSO 4:1	880.92	63.13 ± 2.81***	12.40 ± 0.55
6	**8c**	H_2O/ DMSO 4:1	347.81	23.00 ± 4.10	11.44 ± 2.04
7	**8c**	H_2O/ DMSO 4:1	547.73	28.28 ± 2.65*	8.93 ± 0.84

[a] Absorbed amount was calculated by subtracting the amount of **8** in the donor compartment after 30 min (removed from skin by washing with PBS four times) from the applied amount. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to the corresponding value of RSDL®.

333 ± 226 μg $cm^{-2}h^{-1}$ respectively) (Dalton et al., 2006). In such a case, considerably higher rates of nucleophile permeation would be required in order to neutralize a substantial amount of the depot and minimize intoxication effects. In this regard, the permeation rates of **8a** (621.65 ± 39.45 μg $cm^{-2}h^{-1}$) and **8c** (1181.83 ± 126.79 μg $cm^{-2}h^{-1}$) in H_2O/DMSO 4:1 were found to be significantly higher than those of **12a** and VX. Hence, based on the abovementioned VX absorption rates, skin application of these formulations for 30 min should allow nucleophile permeation at a rate and accumulated amount sufficient to neutralize the expected depot.

The permeation of **8a** in mPEG/H_2O 9:1 was not significantly higher than that of RSDL®, emphasizing the important role of the vehicle solution. Replacing it with water or H_2O/DMSO 4:1 significantly improved the permeation by an order of magnitude and more. High concentrations of the permeate and the use of an organic ion pair (as in **8c**) resulted in further improvement.

As mentioned above, in a realistic scenario, a "catch up" therapy utilizing a scavenger of the type described above will most likely be employed only in the case of intoxication signs appearance, in conjunction with an antidotal treatment. In such cases, a considerable amount of VX will have already accumulated as a dermal depot even before application of the scavenger. Therefore, another significant parameter expected to influence the efficacy of the scavenger, is the amount of scavenger absorbed and accumulated inside the skin (i.e., trapped in the dermal component) within a short time window. In this regard, the amount of **8a, 8c** absorbed in the skin 30 min following application ($A_{0.5}$) was significantly higher using formulations with high permeation rates in comparison to RSDL® (Table 7 entries 5, 7). Furthermore, it is safe to assume that these amounts of nucleophiles would be sufficient to efficiently hydrolyze a finite dose (<10 mg cm^{-2}) of active VX residing in a dermal reservoir.

Last, in regards to practicality, it is worth mentioning that Acetohydroxamic acid (**8**) is of low toxicity (chronic per os daily dose of up to 15 mg/kg/day is approved for clinical use) orphan drug (Lithostat) approved by the FDA for the oral treatment of urinary infections (Lithostat [package insert]. San Antonio, TX:Mission Pharmacel; 2015), and that both DMSO and diethylamine are approved for human use and are currently in use in different formulations of diclofenac.

4. Conclusion

We described here nucleophilic lotions based on approved drugs and vehicles, which may be useful for skin application as a "catch-up therapy" against percutaneous intoxication by low volatility OP nerve agents such as VX, as part of the medical treatment against such agents. These lotions are comprised of acetohydroxamic salts as their active ingredients and a mixture of solvents (H_2O/DMSO) that allow the hydrophilic salt to penetrate through the skin as a vehicle. As the hydrolysis rates of VX of such lotions are similar to that obtained by the contemporary active decontamination solution RSDL®, they are expected to perform a double action of both external and within skin decontamination. In this regard it is noteworthy that although the excised pig skin model may serve as a useful tool, especially in comparative studies of similar lotions of the kind presented in our work, this *in-vitro* model also has limitations. Specifically, as the skin penetration occurs only by diffusion and the integrity of the cells after at least one freeze–thaw cycle is unclear, the data cannot be used to accurately predict the *in-vivo* situation, both in animals and humans. Accordingly, we conducted such *in-vivo* studies in domestic pigs which were exposed percutaneously to VX and were treated with lotions of **8a** and **8c**, combined with standard antidotal treatment. Indeed, in these studies the addition of the above "catch-up therapy" lotions substantially improved the recovery of the exposed animals (manuscript in preparation).

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CRediT authorship contribution statement

Victoria Nahum: Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Uri Nili:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **Eugenia Bloch-Shilderman:** Conceptualization, Methodology, Formal analysis, Resources, Writing - original draft, Writing - review & editing. **Boris Smolkin:** Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Writing - review & editing, Visualization, Supervision. **Nissan Ashkenazi:** Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supporting information contains kinetic measurements (NMR, UV–Vis) for the degradation of VX and other OP's, pK_a measurements and skin permeation experimental data. Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2021.120689.

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