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Sarin simulants show limited representativeness†

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In this study, we conducted colorimetric gas-phase tests on real sarin and compared the results with the most commonly used simulants under identical test conditions. Our findings indicated that reactivity extrapolation was not a reliable approach and that validation using the real toxic gas remained essential for a fair assessment of sarin sensors.

Chemical warfare agents (CWAs) represent a threat to both armed forces and civilians. Consequently, the detection of CWAs is a major area of research.[1–13] Nerve agents, a particularly lethal class of chemical warfare agents, inhibit the acetylcholinesterase enzyme (AChE), leading to death by asphyxia or stroke.[14,15] Sarin, also known as GB, is a G-type warfare agent. It has been used several times in attacks such as the Tokyo subway incident[16] and in Syria.[17] This persistent threat necessitates detection methods that are easy to deploy, rapid and unambiguous, even in complex environments. The development of fast and reliable detection methods is especially urgent, as current techniques suffer from significant limitations, such as lengthy analysis times, the need for sample preparation, limited sensitivity, and high rates of false positives.[18–21] Significant resources have been allocated to the development of new sensors, resulting from advances across a range of technologies. To name but a few, notable progress has been made in optical detectors,[1,2,7,9,22–38] gas chromatography-mass spectrometry,[13,39,40] functionalized transistors,[41–43] and biosensors.[44,45] An overwhelming majority of the reported studies have been carried out using simulants to evaluate the potential of these technologies.[46–49] For instance, while studying optical detection in our lab, among the publications related to the optical detection of nerve agents, only 9 out of 99 used an actual nerve agent in the trials, 68 used DCP, 16 used DFP, and 2 used DMMP (see Table S1†). Moreover, remarkably, only 3 used actual nerve agents in the gas phase.

Indeed, real warfare toxic agents, such as nerve agents, cannot be handled in most labs, as they require specific and rare authorisations, a dedicated environment to address health risks, well-defined decontamination protocols, and highly secure, regulated storage of chemicals. Moreover, the possession and use of toxic substances must be declared to the Organisation for the Prohibition of Chemical Weapons (OPCW) and, of course, be strictly for research purposes.[50] The specific gas-phase applications require even more stringent authorisations and security protocols due to the higher risks of exposure. Consequently, the vast majority of the literature on nerve agent detection relies on the simulants shown in Fig. 1. DCP (diethylchlorophosphate) is commonly used for the gas-phase detection. DMMP (dimethyl methylphosphonate) is primarily used to simulate physical properties of sarin, having a similar chemical composition and vapour pressure but much lower chemical reactivity.[51–53] DFP (Diisopropylfluorophosphate) contains a reactive P–F bond, similar to sarin. Although the inherent limitations of simulants have been discussed by other authors,[54] we present, to the best of our knowledge, the first large-scale vapour-phase quantitative comparison of colorimetric sensor reactivity toward sarin and its most common simulants.

A set of 26 different coloured organic molecules were used as probes (see Table S2†). Twenty-five molecules were purchased and another one, 4,4′-((1*E*,1′*E*)-quinoline-2,4-diylbis(ethene-2,1-diyl))bis(*N*,*N*-dimethylaniline), was obtained through a 3-step synthesis developed in our laboratory.[55] The bunch of commercially available molecules was composed of

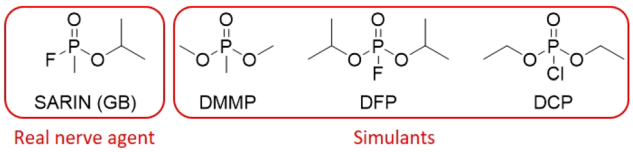

Fig. 1 Chemical structures of sarin, DMMP, DFP, and DCP.

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nine azo compounds, twelve triarylmethane dyes, one anthraquinone derivative, two rhodamine derivatives and one cyanine derivative, all bearing reactive groups. The probes have been selected without any preconceived ideas, with the sole focus being on the colorimetric characteristics and the presence of chemically reactive functions.

The protocol to prepare different samples is as follows: first, each chemical probe was solubilized in DMSO in order to obtain a 10 mM solution. Then, a volume of 3.5 μL of each solution was dropped onto a fiberglass paper. The paper was dried at room temperature for 7 h. The supported probes were then placed inside a homemade sample holder containing 26 holes to allow the gas to pass freely through the paper. Four identical sets containing the 26 papers were prepared. Each of them was exposed independently to vapours of the 3 simulants (DMMP, DFP and DCP) and sarin. Each test set was exposed using the same procedure. The chemicals to be detected were deposited in the form of liquids in excess onto a fiberglass paper at the bottom of a Petri dish. The fiberglass paper was used to enhance the gas diffusion within the test chamber to reach saturated vapour pressure. The sample holder containing the 26 samples was then placed above the fiberglass paper to avoid any contact between the toxicants and the supported probes. The latter were then exposed to the various chemical vapours during 1 h at room temperature under ambient atmosphere in a closed Petri dish to avoid any leakage, particularly of the toxic compounds (Fig. 2). The estimated saturated vapor pressures obtained under the experimental conditions were 2533 ppmv (14.75 mg L^{-1}) for sarin, 128 ppmv (0.92 mg L^{-1}) for DCP, 751 ppmv (5.75 mg L^{-1}) for DFP and 775 ppmv (3.99 mg L^{-1}) for DMMP. Due to high restrictions for real

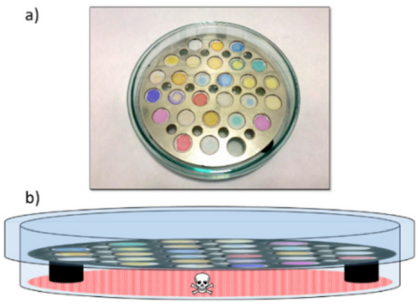

Fig. 2 (a) Image of the test group with 26 colorimetric sensors arranged in the sample holder, placed inside the Petri dish. (b) Schematic of the sample holder within the Petri dish.

Table 1 Colorimetric results of the test group exposed to vapours of sarin, DCP, DFP, and DMMP. Green boxes highlight responses with ΔE_{1994} > 10%

Probe	Ref	Sarin	DCP	DFP	DMMP	Probe	Ref	Sarin	DCP	DFP	DMMP
1						14					
2						15					
3						16					
4						17					
5						18					
6						19					
7						20					
8						21					
9						22					
10						23					
11						24					
12						25					
13						26					

sarin-based experiments, the possible parameters to be changed were unfortunately very limited (just one exposure time and no direct measurement of the toxic concentration).

Pictures were acquired under visible light and scans of the supported probes were recorded before and after exposure using a desktop scanner. During the scan, a black and white sight was used in order to adjust the colours of the scan to be uniform between all the experiments. The colorimetric results are displayed in Table 1.

The colorimetric results were subjected to mathematical calculations in order to eliminate the potential for reliance on the subjective interpretation of colour by the human eye. RGB coordinates were measured at the centre of each spot. They were then transformed into *XYZ* coordinates and then into Lab coordinates. The coordinates before and after exposure were then mathematically compared in order to obtain a percentage from the ΔE_{1994} value defined by the International Commission on Illumination (details are presented in ESI†). The measurements performed before each exposure were used as reference for comparison. The ΔE_{1994} value corresponds to the difference in colour before and after exposure.

A limit value of 10% was chosen to differentiate a positive and a negative response, thus if $\Delta E_{1994} > 10\%$, the change was considered significant and the response was positive to the presence of a toxicant. If ΔE_{1994} was less than 10%, the change was considered insufficiently significant and the response was deemed negative. The main limitation of the method using ΔE_{1994} would be the results close to the limit value of 10%, but out of the 104 calculated values of ΔE_{1994}, only twelve displayed a result between 8% and 12%. Table 2 shows the results before and after exposure to sarin, DCP, DFP and DMMP (see Table S3† for all ΔE_{1994} values).

It was observed that 19% of the molecules change colours after exposure to sarin, 50% after exposure to DCP, only 4% reacted to DMMP and none reacted to DFP. These data point out DCP as the best simulant of sarin for the tested molecules. However, only 31% of the molecules reacting to DCP showed positive sensing to sarin. This is indicative of a high difference in reactivity. This demonstrates that the observation of a positive detection with DCP must not be used to extrapolate a positive result for real sarin. Molecule 20 is the only one that reacted to DMMP, but it did not react with the other simulants or sarin. Molecule 25 showed no reactivity towards any simulant but reacted to sarin, proving again the absolute difference of reactivity between sarin and its simulants.

The reactivity with sarin and simulants of each family of dye is presented in the form of Venn diagrams in Fig. 3. Azo compounds display a good reactivity towards DCP with 67% of reacting probes, but no reactivity with sarin or other simulants

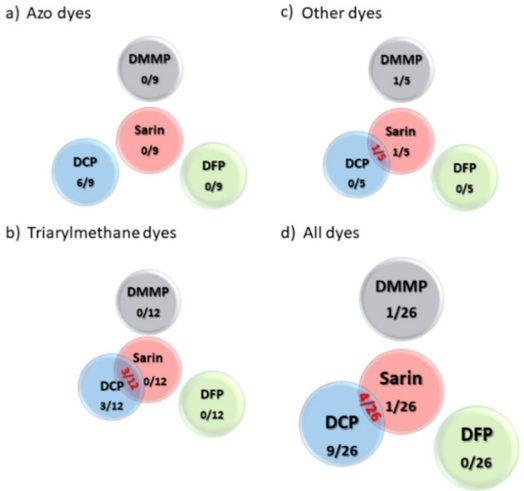

Fig. 3 Venn diagrams showing positive results of (a) the azo compounds only, (b) triarylmethane dyes only, (c) all other molecules, and (d) the entire set of colorimetric sensors. The ratios inside the bubbles represent positive sensing for sarin or simulants, while the values in the overlapping areas between the bubbles indicate compounds that detect both organophosphorus molecules. A detailed table of all the results is presented as Table S4.†

was observed. Moreover, 50% of the triarylmethane dyes reacted with DCP and 25% reacted positively with sarin. The triarylmethane dyes reacting with sarin also showed positive results with DCP. The anthraquinone derivative did not show any reactivity. One of the rhodamine derivatives reacted with DMMP, while the other induced a colour change with sarin. The cyanine derivative displayed no reactivity. The synthesized probe reacted with both DCP and sarin.

Table 2 Positive results from exposure to sarin and its simulants

	Sarin	DCP	DFP	DMMP
Number of positive responses	**5**/26	**13**/26	**0**/26	**1**/26
In percentage	19%	50%	0%	4%

Conclusions

The main output of this study, based on twenty-six coloured molecules and four different organophosphorus reactants in the vapour phase including real sarin, is that extrapolating results for a real toxicant based on the response of simulants are not relevant and must be avoided. Nevertheless, the use of simulants remains the only expedient and straightforward method for obtaining a proof of concept, given the challenging and complex accessibility to authentic nerve agents, in particular in the gas phase.

In conclusion, it is important to keep in mind that the results obtained with simulants present very significant limitations. It is therefore imperative that trials be conducted with a genuine toxic substance in order to validate a gas-phase nerve agent sensing technology.

Author contributions

Valentin A. Bureau: Conceptualization, data curation, formal analysis, investigation, methodology, writing – original draft. Sébastien Penlou: Formal analysis, methodology, supervision,

validation. Sonia Sousa Nobre: Formal analysis, methodology, funding acquisition, supervision, validation. Alexandre Carella: Formal analysis, methodology, supervision, validation. Jean-Pierre Simonato: Formal analysis, supervision, validation, writing – review and editing.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

There are no conflicts to declare.

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