Painful chemistry! From barbeque smoke to riot control

Christopher Green, Farrha B. Hopkins, Christopher D. Lindsay, James R. Riches, Christopher M. Timperley*

Defence Science and Technology Laboratory (Dstl), Porton Down, Salisbury, Wiltshire SP4 0JQ, UK

Abstract

Pain! All humans feel it throughout their lives. The molecular mechanisms underlying the phenomenon are still poorly understood. This is especially true of pain triggered in response to molecules of a certain shape and reactivity present in the environment. Such molecules can interact with the sensory nerve endings of the eyes, nose, throat and lungs to cause irritation that can range from mild to severe. The ability to alert to the presence of such potentially harmful substances has been termed the 'common chemical sense' and is thought to be distinct from the senses of smell or taste, which are presumed to have evolved later.

Barbeque a burger excessively and you self-experiment. Fatty acids present in the meat break off their glycerol anchor under the thermal stress. The glycerol loses two molecules of water and forms acrolein, whose assault on the eyes is partly responsible for the tears elicited by smoke. Yet the smell and taste of the burger are different experiences. It was this eye-watering character of acrolein that prompted its use as a warfare agent during World War I. It was one of several 'lachrymators' deployed to harass, and the forerunner of safer chemicals, such as 'tear gas' CS, developed for riot control. The mechanism of action of some sensory irritants is discussed here in relation to recent advice from the Scientific Advisory Board (SAB) of the Organisation for the Prohibition of Chemical Weapons (OPCW) on chemicals that conform to the definition of a riot control agent (RCA) under the Chemical Weapons Convention.

Introduction

Before discussing modern aspects of our understanding of lachrymators and their historical relevance to RCAs, it is worth recalling the history of chemical disarmament efforts, in which The Hague has special prominence [1]. In this city, Tsar Nicolas II of Russia convened an international peace conference in 1899, where delegates attempted to reach agreement regarding methods and means of conducting war. Among the subjects discussed was chemical warfare (CW), although these words were not the terminology then in use [2]. A final agreement, signed by various nations including Great Britain and Germany, and called the 1899 Hague Convention Respecting the Laws and Customs of War on Land, contained the declaration that all Parties to the Convention agreed to "abstain from use of all projectiles the sole object of which is the diffusion of asphyxiating gases."

Another peace conference followed in The Hague in 1907 and the earlier convention was expanded to include the declaration that "it is especially forbidden to employ poison or poisoned weapons." The expanded Convention (hereafter the "Hague Convention") faltered when Germany, careful to avoid the use of projectiles, used cylinders containing chlorine at the front in Ypres, in Belgium, on 22 April 1915. Thus started the modern age of chemical weapons.

The military use of sensory irritants preceded the use of chlorine. It began in 1910-1914, when ethyl bromoacetate was employed against criminals by the French police [3]. At the start of the First World War, some of the former policemen, by now in the French army, began to use irritants on the battlefield with some success. This gave the British the same idea.

The first step in the initiation of sensory irritant development in Great Britain was made in November 1914 [4] when Professor Herbert Brereton Baker CBE FRS (1862-1935), a member of the Royal Society War Committee, discussed with Professor (later Sir) Jocelyn Field Thorpe CBE DSc FRS (1872-1939) [5] (Note 1) the possibility of obtaining some substance, 'which while itself innocuous, and therefore not barred by the Hague Convention, could cause the atmosphere of a trench or enclosed space to become either intolerable or to so incapacitate the occupants as to render them incapable of effective resistance' [4].

This question had previously been discussed by the Royal Society Committee and the Scottish chemist Sir William Ramsay KCB FRS FRSE (1852-1916) (Figure 1a, Note 2) had suggested the employment of acrolein, a substance the use of which he had previously advocated during the Russo-Japanese War (a war fought between the Russian Empire and the Empire of Japan in 1904-1905 over rival imperial ambitions in Manchuria and Korea). It was pointed out, however, that not only did acrolein require the use of glycerine in its manufacture – a substance which was required for other war purposes – but that it was unstable and readily passed into a useless polymer when kept. Moreover the substance could hardly be described as innocuous since many of those who had worked with it had found that unpleasant throat and lung symptoms were produced by such small concentrations as are usually met with in a laboratory during experiments with it [4]. Experiments were, however, carried out by Professor Thorpe and colleagues but they were unable to find an effective stabilizer. It is of interest to note that this problem was solved subsequently by Professor Charles Moureu (1863-1929) [6] at the Collège de France in Paris.

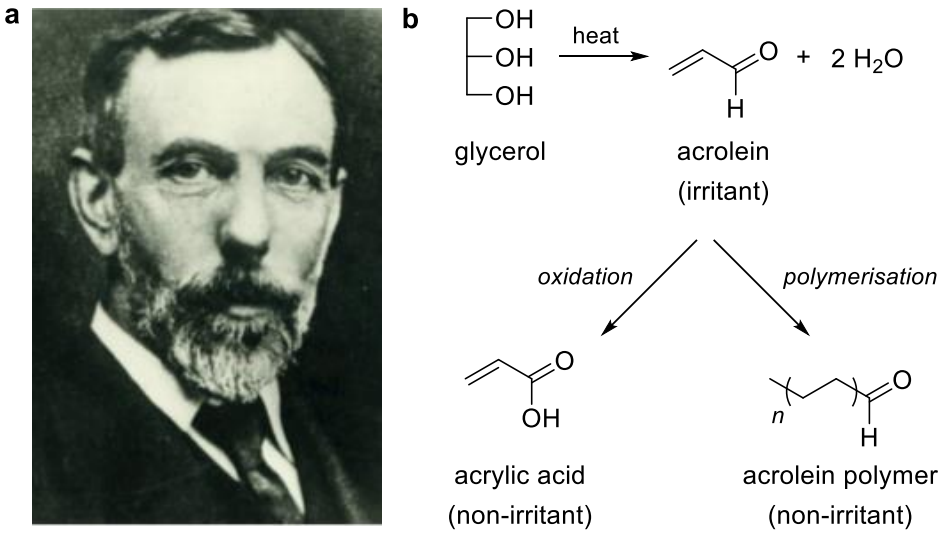


Figure 1. (a) William Ramsay whose suggestion to the British government to use acrolein as a chemical agent during the First World War was dismissed (photograph reproduced by permission of the author C.M.T. from his private collection). (b) Glycerol from overheating fatty meats breaks down to the irritant acrolein, which over time oxidises to the non-irritant acrylic acid or polymerises to give the non-irritant "acrolein polymer". The structure of the polymer as shown is an oversimplification; other polymeric structures also form [11].

Acrolein, a colourless liquid that boils at 52 °C, was discovered originally by Dr Joseph Redtenbacker (1810-1870) [7] (Note 3), while a junior professor at the University of Prague, in 1843 [8]. It was first used as a CW agent by the French in 1916, being suggested by Moureu's co-worker Adolphe Lepape, whence its name of "Papite" [9].

Acrolein was the only aldehyde employed as a CW agent during the war of 1914-1918, and its use was very limited. It was inefficient because of its tendency to oxidise (to acrylic acid, which is non-irritant [10]) and to polymerise into an amorphous white solid: disacryl and/or acrolein gum [11,12]. To retard polymerisation the French added about 5% by weight of amyl nitrate as a stabiliser. While this helped prevent polymerisation to disacryl, it did not suppress the formation of acrolein gum, so it was not very effective [13]. On account of its instability, acrolein was unsuccessful as a CW agent in the First World War, despite being an effective irritant (as experienced in everyday life from smoke particles from overcooked meat). The process of formation of acrolein via dehydration of glycerol, itself produced from the decomposition of glyceride fats, is shown in Figure 1b.

Attention was therefore turned to other substances and it appeared to Professor Thorpe that the solution of the problem seemed to lie rather in the use of eye irritants than lung irritants because the former seemed to be effective in very much higher dilutions [4]. He was led to this conclusion because during his research work at Manchester he had prepared large quantities of *ortho*-xylylene dibromide (**1**) (Figure 2) and had, on one occasion caused the evacuation of the entire university buildings including the private room of the Vice-Chancellor. It was true that this striking result was mainly due to the fact that he had, unknowingly, carried out the experiment in the neighbourhood of the air intake supplying the ventilating system but, still, the amount which passed into the air supply must have been exceedingly small, and the outcome clearly indicated the 'value of the substance for offensive purposes' [4].

Moreover, Professor Thorpe had frequently noticed that these eye-irritants possess the property of being absorbed by woollen clothing from which they are gradually evolved during the course of some hours. On many occasions he had noticed the effect on his fellow

travellers in the railway train on his way home and had listened to their remarks on the character of the tobacco smoke by other occupants [4].

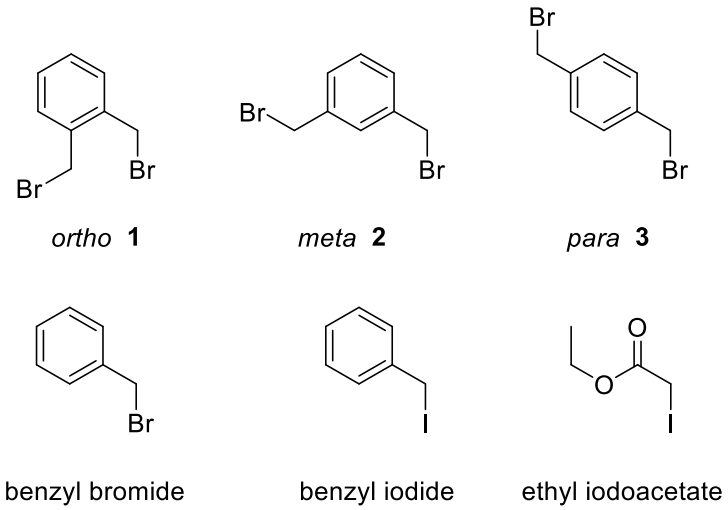


Figure 2. Chemicals tested for lachrymatory properties in the Imperial College trench.

Ortho-xylylene dibromide (**1**) seemed therefore a likely substance for investigation, but, unfortunately it was very difficult to prepare, since *ortho*-xylene did not occur to any appreciable extent in the coal tar distillate. It was found the *meta* derivative (**2**), which is the one most readily obtained, was not nearly so pronounced in its effects [4]. The *para* compound (**3**) was also found to be comparatively ineffective.

The idea, however, of the employment of lachrymators was clearly worth following up and it was decided to carry out an investigation of a whole series of likely compounds [4], Colonel (later General) Jackson A.D.F.W. being associated with the work on behalf of the British War Office.

In all some 50 substances were prepared and experimented with in the trench at Imperial College in London, where Thorpe was then Professor of Organic Chemistry. The process adopted was to burst a glass vessel, containing the material, by means of a detonator, or, in the earlier days, by merely throwing it against the wall of the trench [4]. Observations were made personally by Colonel Jackson, Professor Baker and Professor Thorpe, the plan being to enter the trench at definite intervals after the explosion and to note the lapse of time before

the atmosphere became tolerable. As the trench used was situated in a small quadrangle surrounded by high buildings there was usually complete absence of wind and the comparisons were therefore made under exceptionally favourable conditions.

It was soon found that of the three observers Colonel Jackson was by far the most resistant [4]. He could, in fact, sit down and smoke his pipe in concentrations which were quite intolerable to Professor Baker and Professor Thorpe. He was therefore used a standard and acted in that capacity thereafter. On some occasions he would send his assistant, and Professor Baker and Professor Thorpe and were astonished to find that the assistant was almost as resistant as Colonel Jackson, until they noticed that it was his invariable practice to shut his eyes during the period of exposure.

In the course of time the field was narrowed to three or four possible substances of which benzyl bromide, benzyl iodide and ethyl iodoacetate (Figure 2) were the chief [4]. Chloropicrin (CCl_3NO_2) was found to be 'good' but not so effective a lachrymator as the others. Of the three substances named, the benzyl derivatives were discarded owing to the employment of toluene in their manufacture and the choice fell on ethyl iodoacetate chiefly because it utilized no material of value for war purposes and also because it was the only one of the three which did not attack shell metal – an important consideration when it is remembered that otherwise the steel shell would have had to be provided with a protective internal coating.

The first demonstration of the new substance was held before representatives of the War Office at South Kensington in January 1915 and was entirely successful [4]. The issue was at one time in doubt because one representative was very tall and, with his head well above the trench parapet, asserted that he felt nothing. On the other hand another representative was very short and, with his head well below the parapet, wept copiously. Ultimately Colonel Jackson offered a boy who happened to be passing a shilling if he would walk through the trench. The remarks of the boy settled the question.

Field scale trials were then carried out at Chatham and for this purpose small tins fitted with a detonator sleeve were used. But the testing did not go as smoothly as planned. According to Professor Thorpe, 'it was annoying to find on the morning of the trial that several of the tins had been perforated by the action of the liquid. Consequently the journey to Chatham could not be made by train and the leaky tins had to be strapped at the back of a motor car. The

effect was clearly noticeable in a village in which we had to stop to effect some minor repairs' [4].

The Chatham trials were also successful and were particularly interesting to Professor Thorpe because they illustrated a fact which he had since been able to verify on many other occasions, namely that the subjective effect of chemical materials on inexperienced observers was very great. He noted that during the Chatham trials 'a number of officers from the Garrison acted as observers and one of them who was stationed at least 50 yards up wind from the point of burst immediately left the trench, showing every sign of great mental disturbance and stating that he felt very ill. It was quite impossible that any of the vapour could have gone anywhere near him' [4].

At this stage the question of manufacture was considered because although the chemical artillery shell had not yet been developed it was Colonel Jackson's plan to use large numbers of small canisters containing the liquid and to project them by means of 'catapults and other contrivances' into the enemy trenches [4]. Sir George Thomas Bielby FRS (1850-1924) [14] (Note 4) was therefore called into consultation and it was ultimately arranged that the manufacture should be carried out at the Cassel Cyanide Works in Glasgow (where Bielby was the director) and that Sir Christopher Kelk Ingold FRS (1893-1970) [15] (Note 5), then a student at Imperial College who had conducted laboratory experiments, should be transferred to the Glasgow works.

At the same time, Professor Thorpe believed that a definite opinion was obtained from the Law Officers of the Crown to the effect that the use of an innocuous substance of the type of ethyl iodoacetate (a mixture of it with 25% by weight of ethanol was nicknamed SK, short for "South Kensington"), was not contrary to the restrictions imposed by the Hague Convention [4]. All was now clear for the manufacture and for use from shell and bombs. In March 1915 the first shell – a 4.5-inch Howitzer shell converted from high explosive – was filled and exploded at rest at Shoeburyness. The result showed that the substance could be usefully employed in this way.

In April 1915, however, the Germans, on the Ypres salient, first used chlorine gas from cylinders and shortly thereafter used lethal substances of the type trichloromethyl chloroformate (CCl_3OCOCl) from artillery shells [16]. All restrictions therefore disappeared and after the British authorities had decided to follow the German practice, a small committee

consisting of Colonel Jackson, Sir George Bielby, Professor Baker and Professor Thorpe was constituted by Lord Kitchener in order to find the best means of accomplishing this end [4]. Colonel Dr Arthur William Crossley (1869-1927) [17-20] (Note 6) was subsequently added to the committee as secretary. This was the beginning of the British Chemical Warfare Department.

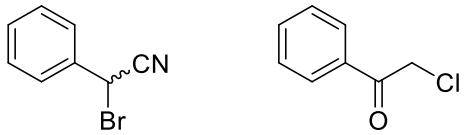
Considerable progress had been made at this stage in the production of ethyl iodoacetate. Although the developments already described had removed the restrictions which caused it to be evolved, the manufacture of the lethal substances of the type of hydrogen cyanide (HCN), phosgene ($COCl_2$) or chloropicrin - which were ultimately introduced - required considerable time. The question therefore arose whether SK as a lachrymator was worth further consideration.

The point was answered in the affirmative because it was recognised that any material which compelled the enemy to wear his mask was useful: the act of wearing the mask reduced considerably his efficiency as a fighting unit [4]. Moreover, the very low concentrations in which the lachrymator was effective – approximately 1000 times lower than that of a lethal substance – enabled this result to be attained by the expenditure of a comparatively small number of munitions. At the same time it was realised that the high boiling point of ethyl iodoacetate (180 °C) combined with its effectiveness at such low concentrations, would cause the effect to persist for some hours after the shell had been fired.

Experiments with ethyl iodoacetate were therefore continued and the conditions under which it would be most favourably employed from shells and bombs were determined. The earlier experiments were carried out at Shoeburyness – ultimately, when the experimental ground at Porton was ready for use, the experiments were conducted there and certain improvements introduced. For example, a new explosive was used for bursting the shells and the 25% by weight of ethanol, which under the earlier conditions of manufacture it was found convenient to dilute the ethyl iodoacetate with, was eliminated and the pure product, referred to as KSK, was introduced.

SK and KSK were largely used in service until towards the end of the war when the introduction of sulfur mustard ($ClCH_2CH_2SCH_2CH_2Cl$) caused its entire replacement. It is, however, of interest to note that while a stronger lachrymator namely bromobenzyl cyanide (military code CA, or in admixture with 30% benzyl cyanide, BBC) (Figure 3) had recently

been introduced by the French, the new substance suffered from the disadvantages which led to the discarding of other benzyl derivatives, namely that it required toluene for its manufacture and had to be contained in glass-lined shells [4].



CA or BBC CN or CAP

Figure 3. Structures of the standard British lachrymators in service after the First World War.

Lachrymators, in the British service in 1919, thus became 'unfashionable, although both the French and the Americans realised that the use of such a substance which is immediately disabling as compared with one which only exerts its effect after a lapse of time after such exposure, has certain advantages from the tactical point of view' [4].

In conclusion it should be noted that SK was evolved entirely as an inhibiting substance without toxic action. When the enemy started the use of toxic gases the British authorities demanded materials which would kill or permanently disable and for these purposes they were provided with hydrogen cyanide, phosgene and chloropicrin. The introduction of sulfur mustard and the large number of casualties which were caused to the British Army before it had discovered the best means of protection led to a reversal of this policy.

The chemistry of the lachrymators of the 1914-1918 War and, in particular, of the two standard British lachrymators, BBC and 2-chloroacetophenone (American codename CN, British codename CAP) (Figure 3), had been studied in detail prior to 1939 and little attention had been directed to these compounds since then. After its discovery by German chemist Carl Graebe in 1871 (1841-1927) [21] (Note 7), the solid 2-chloroacetophenone, observed by Graebe to powerfully irritate the eyes, had been studied extensively by Porton scientists since about the early 1920s, and most of the results had been published in the chemical literature. In consequence relatively little original work on this substance was conducted during the period between the First World War and the Second World War.

The fundamental work performed in Great Britain on lachrymatory substances during the Second World War chiefly concerned the methods of preparation and the chemical properties of these substances, including their physiological potencies. Little attention was paid to the development of new lachrymators in the United States of America or in other parts of the British Commonwealth. The major portion of the work was carried out by an extramural team at the Imperial College of Science under Sir Ian Morris Heilbron DSO FRS (1886-1959) [22,23] (Note 8) and by members of the staff of the Chemical Defence Experimental Station, at Porton. Minor contributions were made from a number of sources, in particular by the extramural team at the University of Oxford under Sir Robert Robinson FRS (1886-1975) [24] (Note 9), and by the Chemical Defence Research Establishment, Sutton Oak, St Helens, not very far from Manchester. The latter establishment, while playing only a small role in the search for new lachrymators made a major contribution on the semi-technical production of lachrymators. The extramural team at the University of Cambridge led by Malcolm Dixon FRS (1899-1985) [25] (Note 10) focused on the biochemistry of lachrymators, particularly their reactions with enzymes and proteins, in an attempt to discover their mechanism of action. (This difficult problem is only now being resolved due to the finding that they interact with certain ion channels, as described later in this article.)

The main object of the British efforts was to discover new lachrymatory substances capable of supplementing/or replacing, wholly or in part, the standard lachrymators BBC and CAP. A secondary objective was to establish, if possible, relationships between chemical structure and physiological action. That lachrymatory activity was usually associated with certain types of chemical structure was well known, and since the above search involved the preparation and physiological testing of many compounds, attempts were made to define more exactly the relationship of chemical structure to lachrymatory potency. To this end, a number of compounds which were unlikely to be capable of production on a large scale were prepared and their irritant potency ascertained.

Among these compounds, certain simple ring-substituted derivatives of CAP were synthesised. Their number was greatly increased at Porton and most of the simple mono- and di-substituted, and some of the tri-substituted, ring derivatives were prepared and examined physiologically. Substitution frequently resulted in a marked diminution or total disappearance of lachrymatory power, and no compound superior to CAP in military properties emerged from this study.

As a result of the research programme on lachrymatory substances it was possible to formulate only imprecise rules relating chemical structure to physiological potency within a given series. This was despite the best attempts of Porton scientists, including the brilliant chemist Arthur Henry Ford-Moore (1896-1958) [26,27] (Note 11), to find relationships between chemical constitution and physiological activity. Doubtless physical factors such as vapour pressure, solubility, both aqueous and lipoid, partition coefficients etc. affect the physiological potency. Unfortunately very few of these physical constants were determined and it was, hence, impossible to subject the results to even semi-quantitative treatment. It was concluded by the Porton workers that only when sufficient data were available to allow for differences introduced by physical factors would it be possible to assess the constitutive element in the relationship between chemical structure and physiological action.

Ford-Moore, admitting defeat, concluded that 'the evaluation of the constitutive effect is largely a matter of luck until the physical characteristics have been determined and their probable effect on physiological activity assessed. For this reason, and on account of the almost total lack of biochemical data in this field, no attempt has been made to suggest a mode of action for these compounds' [28].

Riot control agents

The concept of using non-lethal chemicals to harass or temporarily incapacitate humans in the control of civil disturbances arose from their occasional use by police forces of some nations as an aid to the arrest of violent or dangerous criminals. The first use for law enforcement may well have been in 1912 in Paris; though their greatest earliest use was probably during the 1920s and 1930s in the United States of America. There seems to be no indication of any United Kingdom police force interest in pre-Second World War years.

Since about 1938, CAP had been the most commonly used "tear gas" for flushing criminals from buildings and for dispersing rioters. Its popularity, especially in the United States of America, was due largely to its long-standing availability, its relative cheapness and the ease with which it could be disseminated by pyrotechnics. Additionally, it had been used for many years in the military training of many countries for chemical defence, since it was a useful simulant for unpleasant or lethal CW agents. A commonplace use in this context was respirator testing and in the training of troops in respirator drills, where a modest penalty was incurred by those with ill-adjusted respirators. However, CAP had disadvantages. The

melting point of commercial grade CAP was 51-53 °C and at tropical temperatures, segregation of the charging in pyrotechnic mixtures led to decreased efficiency of emission and an increased risk of leakage in munition storage. Also, the possibility of the liquid phase in the CAP-pyrotechnical mixture was likely to accelerate decomposition of components. Although CAP was a potent lachrymator, its effect was not sufficient to discourage the highly motivated and tolerance was developed on prolonged and repeated exposure. Despite its continued use, it was accepted generally that CAP was not an ideal riot control agent. More seriously, a note of disquiet was introduced by indications that under extreme conditions, transient but significant eye and skin lesions had been recorded in humans and there were hints of more serious possible side-effects.

Both in respect of chemical defence training and riot control uses, it was clear that an alternative to CAP was essential. In 1956, a search for a better agent began at Porton. The required characteristics for a new agent were specified:

- At a concentration in air of 1 part per million (ppm) symptoms should be evoked within 30 seconds and temporary disablement should occur within one to two minutes, to the extent that the rioter is no longer interested in hostile activity and concentrates efforts on escape. Importantly, those exposed must be capable of escape.
- The agent should affect more than one anatomical structure or physiological system. Lachrymation was desirable but this alone, even when accompanied by blepharospasm (rapid blinking of the eyelids), caused real distress. The total effect should transcend high levels of motivation, and morale and should discourage reassembly of crowds.
- The agent should be non-toxic in use and effective at concentrations which did not approach any possible harmful dose for children or aged people who might be accidently exposed. The agent must not cause skin damage or pathological changes in the eyes or lungs.
- The agent should be relatively cheap and readily manufactured. It should have good storage characteristics, be easily disseminated, preferably by pyrotechnic means and be capable of charging into existing munitions.
- Its use must not contravene any international agreements to which the United Kingdom is a signatory.

The search for a riot control agent to replace CAP started with a survey of the available literature and records on structure-irritancy profiles, from which several classes of compound were selected for further study. These included the capsaicinoids (discussed later) and derivatives of benzylidenemalononitrile (BMN) [29] (Figure 4) including 2-chlorobenzylidenemalononitrile [30]. This substance was not identified by the codename CS until 1958, when it was formally accepted by the United Kingdom services as a riot control agent. The designation CS is derived from the first letters of Ben Corson and Roger Stoughton, who first synthesised 2-chlorobenzylidenemalononitrile in the laboratories of Middlebury College, Vermont, United States of America, during academic studies on the condensation products of malononitrile. Corson and Stoughton noted then that the compound had properties akin to a 'sneeze and tear gas' and mentioned the unpleasant effects of handling the powder in their 1928 publication describing the series of compounds that they had synthesised [31].

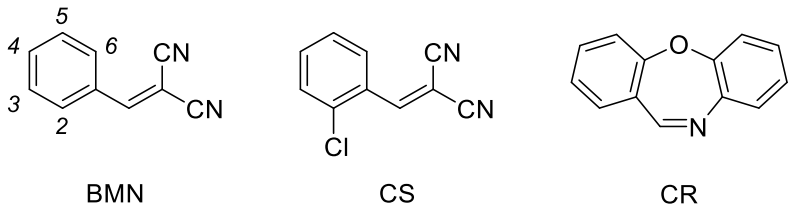


Figure 4. Structures of some phenyl-substituted irritants: benzylidenemalononitrile (BMN) with the numbering system for ring substituents shown, and the riot control agents CS and CR. CS is colloquially referred to as "tear gas" even though it is a solid, like CS and CR, at room temperature.

Further studies showed that 2-chlorobenzylidenemalononitrile could be readily manufactured at little greater cost than CAP, that it was heat-stable, had good storage characteristics and could be effectively disseminated. A comprehensive programme of toxicological work was carried out to confirm the data gathered in the early 1930s (when 2-chlorobenzylidenemalononitrile was first studied at Porton and confirmed to be a potent irritant [32]). This further work showed that 2-chlorobenzylidenemalononitrile was an

eminently safe agent and possessed none of the potentially harmful effects that might ensue from massively excessive exposure to CAP.

After several years of intensive research and development, troop trials under realistic conditions and work on pyrotechnic formulations, the United Kingdom armed forces formally accepted 2-chlorobenzylidenemalononitrile as a replacement for CAP in 1958. The designation CS was henceforth applied to this agent, perpetuating its original 1928 association with Corson and Stoughton. While CS became widely used in the armed forces as a simulant in chemical defence training, it was destined to remain unused for riot control purposes within the United Kingdom until 1969, when it was deployed by the Royal Ulster Constabulary during the riots of 13 and 14 August in Londonderry, in Northern Ireland.

Meanwhile, in the early 1960s, Dr Hans Suschitzky (1916-2012) of the Royal Technical College, Salford, in Manchester, alerted Porton Down of the intense lachrymatory and skin-irritant properties of dibenzo[*b*,*f*][1,4]oxazepine, which he reported later in an open literature publication [33]. It was rumoured that the irritancy was first recognised by the refuse collectors who complained that the laboratory waste contaminated by this stable compound stung the bare hands and caused pain in those areas of skin touched thereafter. The substance was prepared at Porton Down soon after these observations were made and toxicological studies performed carefully. The Porton scientists showed that the substance, later codenamed CR, was highly potent as an irritant but of very low toxicity, making it suitable as a riot control agent [34].

Irritancy and the TRPA1 ion channel

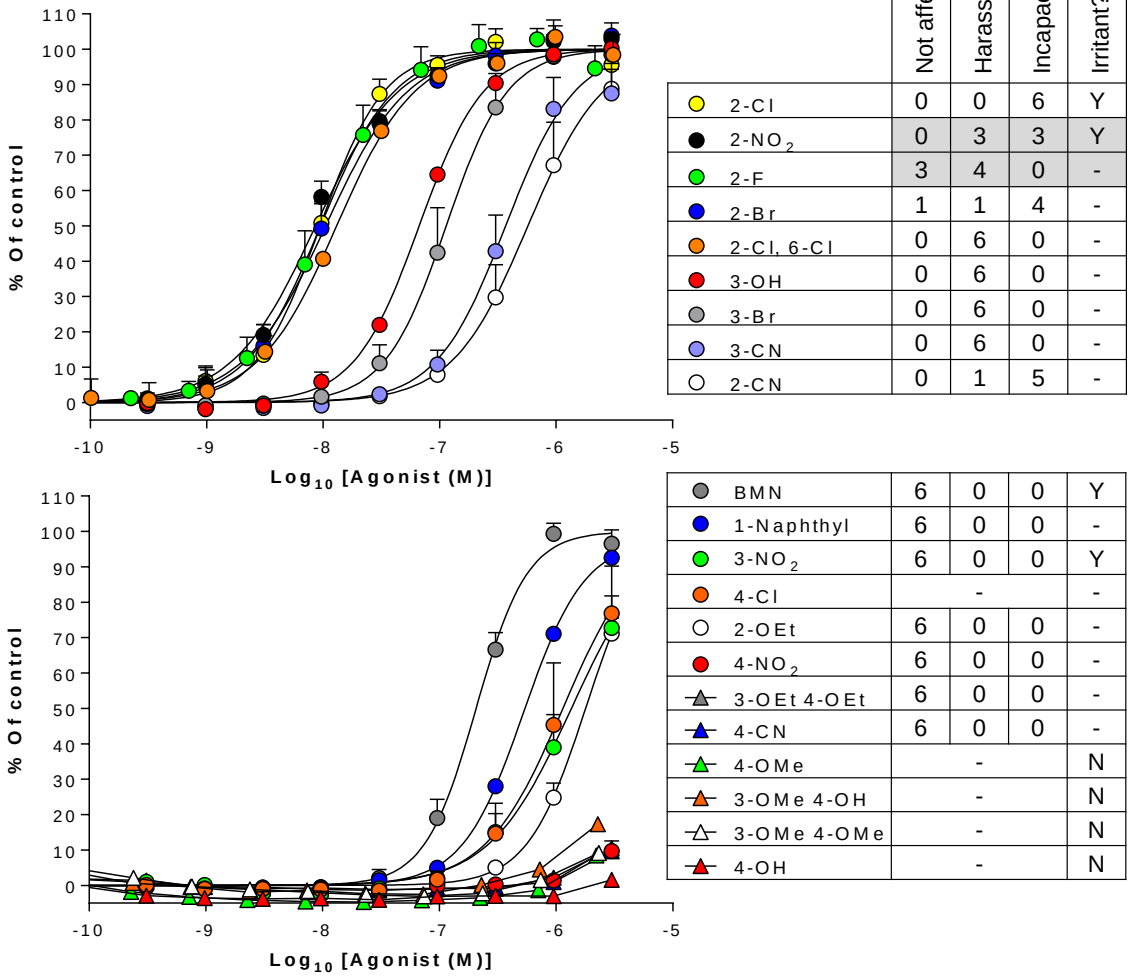
The long-standing mystery of how some of the irritants cause pain was dispelled in 2008 when researchers at Janssen Pharmaceutica in Belgium recognised that the human transient receptor potential ankyrin 1 (hTRPA1) ion channel was a key biological receptor for them [35,36]. In a high-throughput screen aimed at the identification of hTRPA1 ligands, for studying pain induction in humans and options for its treatment, it was discovered that acrolein and ethyl bromoacetate, and the riot control agents CA, CN, CS and CR were potent activators of this ion channel (the latter agents at nanomolar or sub-nanomolar concentrations).

Around the same time, scientists at Yale University, in the United States of America, confirmed that CN, CS and CR activated powerfully hTRPA1, and likewise lachrymators used in the First World War (bromoacetone, benzyl bromide and chloropicrin), and the toxic industrial chemical methyl isocyanate (released during the Bhopal gas tragedy in 1984 that claimed over 2000 human lives) [37]. Thus hTRPA1 is an important ion channel to study due to its potential relevance to the mechanism of action of CW agents and toxic industrial chemicals.

In this connection, the present authors have shown that the physiological activity of solid aerosolised benzylidenemalononitriles including CS in historic human volunteer trials conducted at Porton Down in the 1950s correlates with activation of the hTRPA1 ion channel *in vitro* [38] (Figure 5). This suggests that the irritation caused by the most potent of these chemicals results from activation of this channel. We prepared 50 benzylidenemalononitriles and measured their hTRPA1 agonist properties. All the compounds were synthesised specifically for the sole purpose of seeing if their agonist potency measured by the hTRPA1 channel assay related to their irritancy determined in the historic chamber trials.

A mechanism of action consistent with the physiological activity, involving their dissolution in water on contaminated body surfaces, cell membrane penetration and reversible reaction with a cysteine residue of hTRPAl, supported by data from nuclear magnetic resonance experiments with a model thiol, explained the structure-activity relationships. The correlation provides evidence that hTRPA1 is a receptor for irritants on nociceptive neurons involved in pain perception; thus its activation in the eye, nose, mouth and skin would explain the symptoms of lachrymation, sneezing, coughing and stinging, respectively.

The study was the first to correlate the severity of irritancy to humans from a family of aerosolised chemicals to a mechanism and supports the function of hTRPA1 as a sensor for chemically-induced irritation. It opens the path for further study of other chemicals, including CW agents and RCAs, to see if they also activate this ion channel. Discovering a common mechanism of action of such compounds should aid the development of novel generic drugs to counteract chemically-induced pain, an area of considerable interest to the pharmaceutical industry.



	Not affected	Harassed	Incapacitated	Irritant?
2-Cl	0	0	6	Y
2-NO_2	0	3	3	Y
2-F	3	4	0	-
2-Br	1	1	4	-
2-Cl, 6-Cl	0	6	0	-
3-OH	0	6	0	-
3-Br	0	6	0	-
3-CN	0	6	0	-
2-CN	0	1	5	-
BMN	6	0	0	Y
1-Naphthyl	6	0	0	-
3-NO_2	6	0	0	Y
4-Cl		-		-
2-OEt	6	0	0	-
4-NO_2	6	0	0	-
3-OEt 4-OEt	6	0	0	-
4-CN	6	0	0	-
4-OMe		-		N
3-OMe 4-OH		-		N
3-OMe 4-OMe		-		N
4-OH		-		N

Figure 5. hTRPA1 agonist potency correlates with irritancy to humans. Left panels show concentration-response relationships for the indicated analogues of BMN. Right panels show results from historic human volunteer chamber trials with the analogue dispersed at 1 ppm or 0.1 ppm (shaded) and exposure lasting ~1-3 min. Results show numbers of volunteers (6 or 7) in the indicated categories [30]. Human irritancy of BMN and its analogues described in the literature [31] is also shown (Irritant?). Blanks indicate the compound was not tested. The 4-Cl analogue is included as it was synthesised by Crichton *et al*. [30] but did not proceed to chamber trials at Porton due to a lack of irritancy in initial tests. The discrepancy between the powerful physiological effect of the 2-CN analogue and its low hTRPA1 agonist activity – the only anomaly in our correlation – may arise from a rapid rate of hydrolysis, which diminished its concentration during the cell-based fluorescence assay. (Figure reproduced from ref. 38).

Irritancy and the TRPV1 ion channel

One irritant familiar to many people and associated with pain is capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) (Figure 6). It is a member of a family of compounds called capsaicinoids in reference to their natural source: *Capsicum* chilli peppers. The hot flavour of chillis is due to the presence of a group of seven closely related compounds, but capsaicin and dihydrocapsaicin (in which the alkene double bond is hydrogenated) are responsible for approximately 90% of the pungency. Chilli hotness is measured in Scoville Heat Units (SHU) [39], originally a subjective measurement, but today usually determined by analysis of the capsaicinoid content of the chilli through high-performance liquid chromatography: the conversion generally accepted is that 15 Scoville units equates to 1 ppm capsaicin plus capsaicinoids [40].

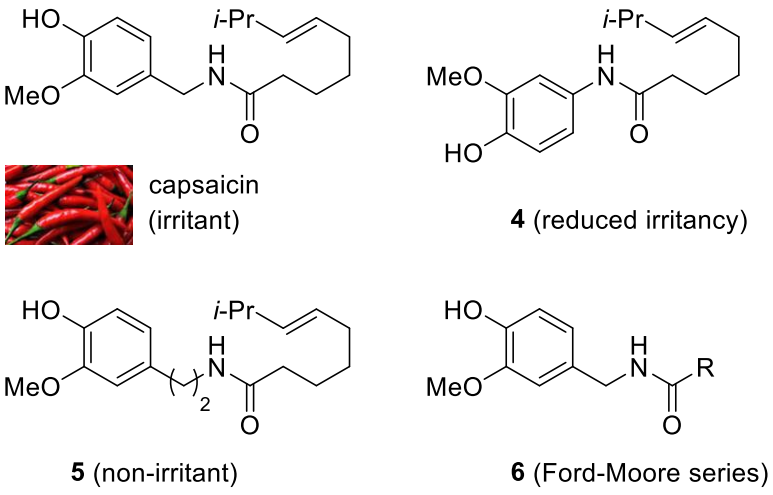


Figure 6. Capsaicin from chilli peppers is a pungent irritant whose effects are well recognised. Modifications to its structure generally produce less active derivatives. Compounds **4** and **5**, in which the benzyl methylene group was removed or extended respectively, were studied at Porton Down in the 1930s and shown to be comparatively innocuous. Substances of structure **6**, where the R group was an alkyl chain, were evaluated also, and maximum activity found when R contained 8 carbon atoms.

Capsaicinoids were exhaustively investigated at Porton during the 1930s, following the pioneering synthetic and pungency studies of E. K. Nelson of the Essential Oils Laboratory of the Bureau of Chemistry, US Department of Agriculture, Washington DC, a decade earlier [41]. Their activity is of a very specific character and almost any alteration in the vanillylamine unit leads to reduced irritancy. Thus, methylation of the *para* hydroxyl group (-OH to -OMe) or demethylation of the methoxy group (-OMe to -OH) reduces the activity enormously. Amides derived from benzylamine are practically inert. Likewise, in the case of the corresponding derivatives of 4-hydroxy-3-methoxy aniline (**4**) and of *beta*-(4-hydroxy-3-methoxy phenyl)ethylamine (**5**) (Figure 6), the former showed reduced pungency and in the latter the pungency is eliminated.

The activity also varies over an enormous range with the number of carbon atoms in the group R in structure **6** (Figure 6). Arthur Henry Ford-Moore and John William Cole Phillips confirmed in the early 1930s that the pungency of vanillylamides reached a maximum at the nonoyl derivative [42]. This series served as an illustration of the difficulty of assessing physiological activity and the need for the use of absolutely standardised methods of procedure. Two methods were used by the Porton chemists for the evaluation of the pungencies depending in each case on the limiting dilution at which reaction was experienced. In the first case two drops of 'standard size' were placed on the tongue and in the second 5 millilitres of solution were 'held in the mouth'!

Capsaicin is difficult to synthesise, other than in very small batches, in a reasonable state of purity and is generally unstable to heat [43]. For these reasons the British government discounted it during its search in the mid-1950s for a riot control agent to replace CAP. It is now known that the mechanism of irritancy of capsaicin occurs through it binding to the transient receptor potential voltage-gated 1 (TRPV1) ion channel [44], which is related in structure to that of TRPA1 [45].

Riot control agents and the Chemical Weapons Convention

Sensory irritants such as the riot control agents already described are chemicals characterised by a very low toxicity, rapid onset, and short duration of action [46]. In general, these agents have a very wide margin of safety [47]. CS is the most widely used compound worldwide for riot control purposes. CN is also used in some countries to control riots despite its higher

toxicity. CR is a more modern irritant but there is little experience of its use. The naturally occurring substance oleoresin capsicum (pepper spray), a mixture of capsaicinoids in which capsaicin predominates as the major pungent component, may find increased use for law enforcement and riot control [47]. Pepper spray is currently available over the counter for personal protection. Other capsaicinoids include *N*-vanillylnonanamide (PAVA). This substance occurs in low concentrations in some *Capsicum* species, but it is synthesised for riot control purposes.

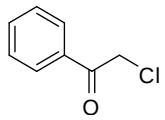
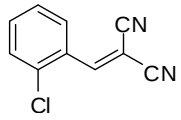
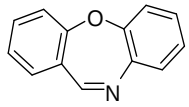
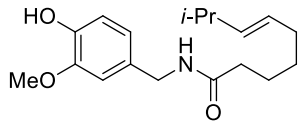
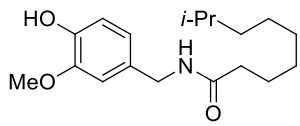
The Chemical Weapons Convention (CWC) [48] was opened for signature on 13 January 1993 and entered into force on 29 April 1997. The treaty is implemented by the OPCW in The Hague. Currently there are 192 States Parties; the United Kingdom of Great Britain and Northern Ireland is one. The CWC prohibits the development, production, stockpiling, acquisition and use of chemical weapons and requires States Parties to destroy, within specific time frames, any chemical weapons and related production facilities they possess.

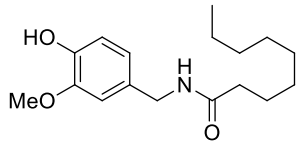
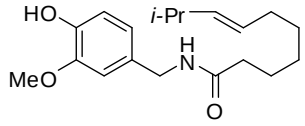
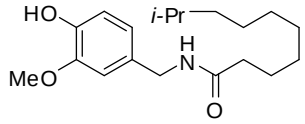
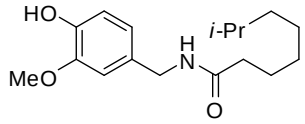
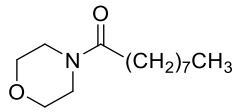
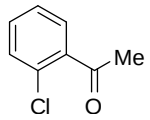
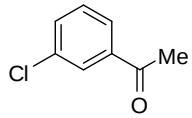
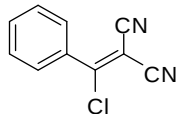
RCAs were a topic of long and heated debates during CWC negotiations. At issue were their inclusion in the treaty and other restrictions that would be imposed upon their use. In the end a compromise was reached under which States Parties are to declare to the OPCW the RCAs they possess for law enforcement purposes. Though use is allowed for these purposes, it is prohibited as a method of warfare.

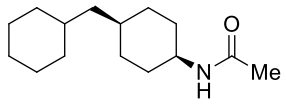
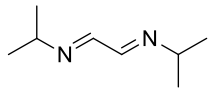
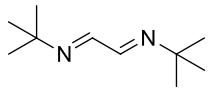
In accordance with subparagraph 1(e) of Article III of the CWC, States Parties are required to declare RCAs, which are defined in paragraph 7 of Article II of this convention as: “Any chemical not listed in a Schedule, which can produce rapidly in humans sensory irritation or disabling physical effects which disappear within a short time following termination of exposure” [48].

At its Twentieth Session, the OPCW Scientific Advisory Board (SAB) [49] was requested by the Director-General to provide technical advice on an initial list of RCAs that had been declared by States Parties, researched, or were commercially available [50]. The SAB advised the Director-General that 17 chemicals correspond to an RCA as defined by paragraph 7 of Article II of the Convention [51]. These substances, their Chemical Abstract Service (CAS) numbers, and available melting point (mp) and boiling point (bp) data, are listed in the Table.

Table. List of 17 chemicals that the OPCW SAB advised the OPCW Director-General corresponded to an RCA as defined by paragraph 7 of Article II of the CWC [51].

Chemical name	**CAS number**	**Physical state**
2-Chloroacetophenone (CN) 	532-27-4	White solid Mp 54-56 °C Bp 245 °C
2-Chlorobenzylidenemalonitrile (CS) 	2698-41-1	White solid Mp 93-95 °C Bp 310-315 °C dec.
Dibenzo[*b*,*f*][1,4]oxazepine (CR) 	257-07-8	Yellow powder Mp 72 °C Bp 335 °C
Oleoresin capsicum (OC) Resin containing ≥8% capsaicins: capsaicin, dihydrocapsaicin, and nordihydrocapsaicin.	8023-77-6	A waxy resin
8-Methyl-*N*-vanillyl-*trans*-6-nonenamide (capsaicin) 	404-86-4	White solid Mp 62-65 °C Bp 210-220 °C/0.01 mmHg
8-Methyl-*N*-vanillylnonanamide (dihydrocapsaicin) 	19408-84-5	White solid

N-Vanillylnonanamide (PAVA) 	2444-46-4	White solid Mp 57 °C
N-Vanillyl-9-methyldec-7-(*E*)-enamide (homocapsaicin) 	58493-48-4	Crystalline or waxy solid.
N-Vanillyl-9-methyldecanamide (homodihydrocapsaicin) 	20279-06-5	Crystalline or waxy solid.
N-Vanillyl-7-methyloctanamide (nordihydrocapsaicin) 	28789-35-7	Crystalline or waxy solid.
4-Nonanoylmorpholine (MPA) 	5299-64-9	Liquid Bp 310 °C
2'-Chloroacetophenone 	2142-68-9	Colourless liquid Bp 229 °C
3'-Chloroacetophenone 	99-02-05	Colourless liquid Bp 228 °C
α-Chlorobenzylidenemalononitrile 	18270-61-6	White solid Mp 68-70 °C Bp 126 °C/0.1 mmHg

Cis-4-Acetylaminodicyclohexylmethane 	37794-87-9	White solid Mp 112 °C
N,*N*′-Bis(isopropyl)ethylenediimine 	*E*,*E* 28227-41-0 *Z*,*Z* 185245-09-4	Tan-coloured solid Mp 48-50 °C
N,*N*′-Bis(*tert*-butyl)ethylenediimine 	30834-74-3 *E*,*E* 28227-42-1	White solid Mp 39-43 °C

Conclusions

This review has shown that the observations of the irritant action of some chemicals on the human senses led to the development of lachrymators and then riot control agents. The mechanisms of the action of many of these compounds are only now being understood thanks to advances in chemistry and the life sciences; the compounds are being researched as tools to understand better chemically-induced pain. Thus, the studies of chemical constitution and irritancy, so fundamental to a better comprehension of the mechanism of chemically-induced pain, should benefit civilian society by accelerating the discovery of new painkilling drugs.

Notes

1. Biographical details for Jocelyn Thorpe feature in ref. 5. A scientist that worked at Porton stated: ‘Professor Jocelyn Thorpe, who looked more like a brewer’s drayman (except for his eight inch cigar) than a professor of chemical engineering; who had a wonderful private grapevine in industry and was instrumental in engaging one member of the Porton staff to drive a Bentley round Britain on Castrol as a holiday job’ [52].

2. William Ramsay (1852-1916) attended the Glasgow Academy and then the University of Glasgow. He moved to the University of Tübingen in Germany under the supervision of Wilhelm Rudolp Fittig to complete his doctoral thesis on toluic and

nitrotoluic acids. He returned to Glasgow as an assistant to Thomas Anderson. In 1879 he was appointed Professor of Chemistry at the University College of Bristol. The same year he became the Principal of the University College, and managed to combine that role with active research in organic chemistry and on gases. In 1887 he succeeded Alexander Williamson as chair of chemistry at University College London. There he undertook research that led to the discovery of the noble gases in air. This culminated in the award to Ramsay of the Nobel Prize for Chemistry in 1904.

3. Joseph Redtenbacher (1810-1870) was born in Kirchdorf, in Upper Austria, on 12 May 1810 [6]. His father, a merchant, gave him careful education, first at the Stiftsgymnasium of Kremomünster, afterwards at the University of Vienna. In 1834 he took his degree of Doctor of Medicine, and soon after became an assistant to Baron Taquin, professor of botany and chemistry. After passing his examination as a teacher, he went, in 1838, as professor to the Chirurgical Academy at Salzburg, received a travelling fee, and visited the University at Giessen, where he worked with Justus von Liebig, and became acquainted with Robert Bunsen, Augustus Wilhelm von Hofmann, John Stenhouse, Hermann Kopp, and other famous chemists of that era. After his return he was appointed as a junior professor at the University of Prague, and in 1849 he was called to the High School of Vienna, where he worked until his death, which occurred on 5 March 1870.

4. George Bielby (1850-1924) [14] was born in Edinburgh, educated at Edinburgh Academy and Edinburgh University. He joined the Oakbank Oil Company in 1869 where he and William Young increased the yield of oil and other chemicals from shale by retort and fractional distillation improvements. He patented a production method for hydrogen cyanide from ammonia and coal. He was director of the Cassel Cyanide Company and then became the director of the Castner-Kellner Company at Runcorn. He was President of the Society of the Chemical Industry (1899), of the chemical section of the British Association (1905), of the Institute of Chemistry (1909-1912), and of the Institute of Metals (1916-1918). He was knighted in 1916.

5. Christopher Ingold (1893-1970) started his chemical studies at Hartly University College at Southampton (now Southampton University) taking a BSc in 1913 with the

University of London. After a short time with Imperial College, in London, and war service as a scientist, he earned an MSc, again with the University of London. He returned to Imperial College to collaborate with Jocelyn Field Thorpe, and achieved a PhD in 1918 and a DSc in 1921. His pioneering research in the 1920s and 1930s on reaction mechanisms was responsible for the introduction into mainstream chemistry of concepts such as electrophile, nucleophile, inductive and resonance effects. Ingold received the Royal Medal of the Royal Society in 1952 and a knighthood in 1958.

6. Arthur William Crossley (1869-1927) [17-20] was educated in Germany under the tutelage of Emil Fischer and Augustus Wilhelm von Hoffman (where he gained his PhD) and then at Owens College in Manchester UK, undertaking research projects with William Perkin Junior. After several teaching positions, he was appointed professor of chemistry at King's College London in June 1914. This appointment was shortened by the outbreak of war a month later. During the war, Crossley was a volunteer at the British War Office working under Colonel Sir John Pringle. Aiding the large-scale production of the drug salvarsan, he became the secretary of a war committee established by the Royal Society to organise the production of local anaesthetics and other drugs previously only accessible from enemy sources. When the Germans used gas as a weapon from 22 April 1915, the Scientific Advisory Committee and the Commercial Advisory Committee were founded to provide materials to combat this means of warfare. Crossley was appointed secretary of both committees. His success in this capacity, as a communicator of requirements between those working at home and those on the front, led to his appointment as liaison officer for chemical warfare in November 1915, with the rank of lieutenant colonel. He visited several times the French battlefields. In the summer of 1916, the British government realised that the successful development of chemical warfare needed a large experimental testing ground at home. Crossley was entrusted to oversee the suitable conversion of land purchased in Porton, near Salisbury. He resided at Porton for the next two and a half years by which time it was staffed with 47 officers, 700 non-commissioned officers, and 800 civilian workmen – the outcome being to advance the methods by which the effects of poisonous chemicals could be counteracted. For his wartime services he was awarded the CBE, CMG, and appointed an officer of the Legion of Honour. Once the war ended, Crossley held

positions at King's College London and the British Cotton Industry Research Association. He was president of the Royal Society of Chemistry from 1925 to 1926.

7. Carl Graebe (1841-1927) was born in Frankfurt on the Main [21]. He studied under Bunsen at Heidelberg, where he obtained his doctorate in 1862. He conducted his work on the constitution of naphthalene while working at Baeyer in Berlin. Later, as a professor in Königsberg, he studied the higher boiling fractions of coal tar and discovered acridine (with Caro), carbazole (with Glaser), and pyrene. He also researched the structure and synthesis of phenanthrene and chrysene and with Caro he helped elucidate the structure of the triphenylmethane dyes. In 1871, he discovered 2-chloroacetophenone, developed later by others as a riot control agent and codenamed CN or CAP, which he described as having a penetrating smell and powerful irritant effect on the eyes [21]. From 1878 to 1906, Graebe, then a professor at Geneva, continued to advance heterocyclic chemistry, aided by his pupils Ullmann, Kehrman, and Pictet. In 1907 Graebe was elected President of the German Chemical Society. He died in 1927 aged 87 in poverty and almost forgotten, in Frankfurt, where he had lived since retirement.

8. Sir Ian Morris Heilbron (1886-1959) [22,23] served Great Britain with distinction during the two world wars. He joined the Territorial Army in 1910 and was on active service during the First World War, being promoted to the rank of lieutenant colonel as an assistant director of supplies in Salonika, in Greece. Throughout the Second World War, he served as a scientific advisor in London, first to the director of scientific research at the Ministry of Supply and later to the minister of production. Named three times in despatches, he was awarded the Distinguished Service Order, the Greek Order of the Redeemer, and the Medaille d'Honneur in 1918, and the American Medal of Freedom in 1947. He was knighted in 1946 and served as President of the Royal Society of Chemistry from 1948 to 1950. He was an expert organic chemist and conducted important research on vitamins A and D, in addition to studying penicillin and steroids, and helping the development of the insecticide DDT.

9. Sir Robert Robinson (1886-1975) [24] was a giant among chemists. He occupied a large number of important positions in various centres and conducted a huge amount

of research on diverse topics in organic chemistry. The span of his published research was seventy years, in which he attacked increasingly difficult organic chemistry problems, often successfully. He was awarded the Nobel Prize for Chemistry in 1947 for investigations on plant products of biological importance, especially the alkaloids. He consulted for the British Ministry of Supply during the Second World War.

10. Malcolm Dixon (1899-1985) [25] achieved international distinction for his research on physical biochemistry, notably enzyme purification and the kinetics of enzyme-catalysed reactions. He played a leading role in the introduction of the present systematic nomenclature of enzymes. During the Second World War he established under the Chemical Defence Research section of the British Ministry of Supply an extramural research team within the Biochemistry Department of the University of Cambridge to research antidotes to chemical poisons. During the existence of this team, from 1939 to 1945, his 'direction of the war research was a curious mixture of *laissez faire* and a complete grasp of the immediate problems and the way to proceed. Often we (his team) would not see him for days, or even weeks, at a time. This was particularly true if we had results which were difficult to interpret, and were uncertain what to do next. When he did reappear the problem would have been solved, often with a detailed kinetic treatment meticulously set out in his small, neat handwriting on many sheets of paper, or occasionally on long lengths of toilet paper' [25]. The results of the research were provided in a series of 33 papers, known as the "Dixon Reports", to the Ministry. Much of this work was published openly after the war ended.

11. Arthur Ford-Moore was one of the most brilliant chemists that worked at Porton Down, spanning the period 1921 to 1958. His scientific output was prodigious and if it were not for the secrecy surrounding much of his research, he would have been recognised as one of the leading experimentalists of his day. Further biographical details are available elsewhere [26,27]. A colleague that worked with him at Porton commented: 'Arthur Ford-Moore, the organic synthesist and performer of Chopin, generally known as "Uncle Arthur". The more beer he drank, the better became his pianism and his synthesis' [52].

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