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DEVELOPMENT OF A VEGETATION-DAMAGE INDICATOR AS A MEANS OF POST-ACCIDENT INVESTIGATION FOR CHLORINE RELEASES

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Summary

Risk assessment of major hazards involves a wide range of needs to specify the response of receptors to various kinds of harmful exposure. This requirement ranges over many different types, routes and targets of exposure, including for example the response of structures and people to overpressures from explosions, the response of people to thermal radiation from fires and deflagrations, to ionising radiation from radioactive materials, and to systemically and locally acting toxic substances. Within this last broad category, the acute inhalation toxicity of irritant gases is of particular importance, with chlorine as a specific example of special concern. In this paper we examine the problems involved in specifying the population response to acute exposure to chlorine, and the difficulty of selecting suitable reference exposures for risk assessment and related purposes. We then report results of experimental investigations of the response of a common weed species, *Trifolium repens c.v. Huia* (white clover), to controlled exposures to chlorine gas. These experiments were carried out with a view to the use of such vegetation damage indicators as a means of post-accident investigation. Recommendations for the application of such a method of accident investigation are made, based on the experimental findings. Application of this method of investigation could be expected to provide useful information bearing on two significant areas of uncertainty, namely the dispersion of denser-than-air gases, and human toxic response.

General consideration of the need to specify response at various levels of effect

In the general context of major hazards risk assessment the main area of concern evident in published studies (such as the two Canvey Island reports, [1] and [2], and the Rijnmond Report [3]) has been the specification of off-site exposures resulting from accidental releases, at levels likely to cause irreversible injury or death to members of the public. Relatively little emphasis has been given to lower levels of effect, such as the loss of amenity due to the nuisance of relatively frequent small releases (which may occur in normal operation, as well as by accident). The level of exposure and associated response that is appropriate for consideration depends on the role of the organisation requiring the assessment. Thus, a developer considering building a leisure park in the vicinity of a major hazard complex may find considerations of financial

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viability to be the more immediate priority, and therefore will need to have an assessment of the amenity aspect, with associated low levels of exposure. In contrast, the concerns of emergency response planning will necessarily emphasise the mitigation of exposures that might cause death or injury.

The need to consider exposure – response relationships in a regulatory context has emerged in the implementation of the European Community Directive 82/501 and its amendment 87/216 ([4] and [5]), which broadly speaking apply to chemical process plant operations and associated storage. For plants qualifying for the full set of regulations there is a requirement for the preparation of on-site and off-site emergency plans, and the provision of information to persons off-site who are liable to be affected by a major accident. The judgement as to the extent of the area in which people off-site may be affected is a matter for the competent authority appointed under Article (8) of the Directive.

In Great Britain the requirements of the Directive and the amendment are implemented under the Control of Industrial Major Accident Hazards (CIMAH) regulations ([6] and [7]), with the Health and Safety Executive (HSE) as the competent authority. The HSE have issued a guidance note [8] on emergency plans, which states that for toxic releases

> *"Manufacturers should be able to estimate the concentrations and durations of the gas clouds at various distances downwind of the release point. This information may then be used with hazardous dose data to deduce the distances at which toxic effects might be expected and hence the area in which appropriate emergency measures might be needed."*

This item of guidance refers to specific aspects of dispersion and exposure estimation that are part of the techniques of quantitative risk analysis exemplified in the Canvey Island and Rijnmond reports referred to above. Although the Directive does not specify the use of these techniques, it will often be the case that they provide the most appropriate means of establishing the relative likelihood of accidents, and of estimating the extent of areas affected to a specified level for emergency planning purposes. However, there is a need for more specific guidance on the choice of exposure–response reference levels to be used. This is an area of special difficulty, with a wide range of possible interpretation. In the following sections this difficulty is examined by particular reference to chlorine. Although many of the considerations apply broadly to many other substances, chlorine has been chosen as the specific example for several reasons, as follows: it is one of the most widely used of the chemicals of importance in major hazards, and is specifically identified in the E.C. Directive; its physical properties result in its atmospheric dispersion behaviour being that of a denser-than-air gas, with consequent emphasis on this important class of dispersion behaviour; it is a highly toxic irritant gas; there has been a relatively large number of accidental releases (compared to other major hazard gases); preliminary investigations indicated the feasibility of using vegetation damage as a quantitative indicator of exposure.

Chlorine toxicity

The effects of human exposure to chlorine are well known insofar as the nature of the toxic response is concerned. However, there is substantial divergence of opinion as to the quantitative relationship between exposure — in terms of concentration and duration — and the percentage incidence of acute health effects in the exposure population. Chlorine gas is strongly irritant to the skin, and especially to the eyes, nose, throat and respiratory system. As a liquid, chlorine can cause burns and ulceration. The current limits in the U.K. for routine occupational exposure to the gas are 1 ppm for the 8-hour TWA, and 3 ppm for the 10-minute STEL [9].

The literature shows reasonable consistency as to the concentration expected to cause coughing, at about 10 to 30 ppm after 1 minute (see for example [10] and [11]). The principal discrepancies arise for exposures that could be expected to cause injury and death, and which are of particular concern for emergency response. It should be noted that estimates of off-site exposures for accidental releases are sometimes extended to dispersion distances at which the concentrations have fallen to the levels specified in occupational exposure limits. Such estimates can only be regarded at best as being of minor interest, in that whilst they indicate distances at which no symptoms should be expected, they do not illuminate the problem of how to identify the likely extent of areas in which acute effects may occur.This minor role is to be expected given that the considerations underlying the establishment of occupational exposure limits are quite different from those applying to acute symptoms. For example, long-term carcinogenicity and the role of natural metabolic detoxification mechanisms are important for occupational exposures, whilst for acute exposures, which are usually of quite short duration but at concentrations such that metabolic detoxification mechanisms are likely to be utterly overwhelmed, these considerations are of little relevance.

For acute effects the appropriate form of expression is a relationship between concentration, C, duration of exposure, t, and percentage incidence of a specified health effect. Chlorine is one of a number of substances for which the response is dependent on a grouping of the form $C^n t$, where n is an index greater than 1. The varying susceptibility of the exposed population is appropriately expressed in the framework of probit analysis, a statistical technique originally developed to summarise data on the effectiveness of insecticides. The statistical basis is fully described in the text by Finney [12]. For cases where the probability distribution of the incidence of harm follows a log-normal distribution, the probit is related to the percentage incidence of the specified effect as follows: the probit value P is given by

$$P = a + b \ln (C^n t) \qquad (1)$$

where P is a normally distributed variable with a mean of 5 and a standard

deviation of 1, so that the percentage incidence is expressible in units of standard deviation from the mean. Accordingly, for 50% response $P=5$, for 16% $P=4$, for 98% $P=7$ and so on (Table 1 in Finney [12] tabulates this equivalence). In terms of the parameters of the normal distribution curve, $a=5-(\mu/\sigma)$ and $b=1/\sigma$, where μ is the mean and σ the standard deviation. A probit expression in the form of eqn. (1) thus specifies the position of families of percentage incidence lines with slopes of $-1/n$ on a graph of ln C (ordinate) versus ln t (abscissa). Thus, a line representing a 5% incidence joins points for which the probit value is 3.36, and for which the parameter $C^n t$ has a particular constant value.

Two such families of lines are shown in Fig. 1 using probit expressions for chlorine from the industrial comments section of the Rijnmond Report [3] which gave

$$P=-11.4+0.82\ln(C^{2.75}t) \tag{2}$$

and from Purdy et al. [13], who gave

$$P=-4.4+0.52\ln(C^{2.75}t) \tag{3}$$

In both cases the effect level specified is death resulting from chlorine gas inhalation for a human population, and the units are ppm (by volume) for C and minutes for t. These two probit expressions are selected from numerous statements in the literature on chlorine toxicity. The paper by Purdy et al. [13] is of particular interest, as it was written by HSE staff. In an earlier paper

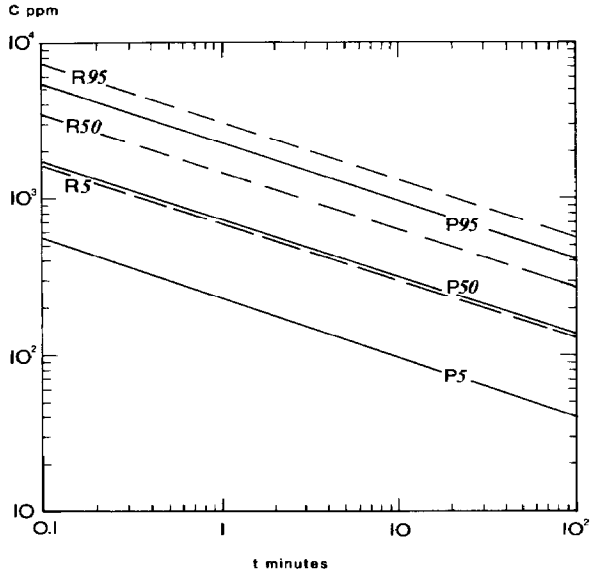

Fig. 1. Chlorine toxicity relationships at the 5%, 50% and 95% levels; P - from Purdy et al. [13], R - from Rijnmond Report Industrial Comments [3].

on chlorine, also by HSE staff, Davies and Hymes [14] reported that the HSE used the relationship $C^{2.75}t = 3.2 \times 10^6$ (C in ppm, t in minutes) for assessment purposes in relation to their advice on land use planning. This quantity was referred to as a significant dose, described by the authors as "a toxicological relationship for a typical human population which would result in distress and a need for medical attention, but is unlikely to result in more than a small proportion of fatalities". It should be noted that the above dose given by Davies and Hymes in fact corresponds to a 5% mortality level in Purdy et al.'s probit expression.

In an examination of the implications of such differences Griffiths and Megson [15] used four available toxic response relationships for chlorine in order to estimate corresponding hazard ranges for a number of release scenarios using a single dense gas dispersion model. The predicted hazard ranges differed substantially over the different toxicity statements, e.g. from 1 to 5 km for the 50% mortality level for a 50 tonne release in Pasquill category D stability conditions with a 5 m/s windspeed.

Figure 1 typifies the problem of selecting an appropriate toxic response relationship from the variety of opinion in the literature. Several features of the two families of lines are worthy of particular comment. Noting the relationship between the probit coefficients a and b, and the means and standard deviations of the distributions, it is expected, since eqn. (3) has the smaller mean but the larger standard deviation, that the two equations will agree at some high percentage mortality. In fact this occurs at the 99.7% level, which for example yields $C = 2100$ ppm at $t = 10$ minutes for both equations. However, the divergence of view becomes quite marked at lower percentage mortalities. Thus, the 50% mortality line from Purdy et al. is approximately the same as the 5% mortality line from the Rijnmond Report industrial comments equation, and the 1% level in the latter view corresponds to a 31% level in Purdy et al.

The problem of chlorine toxicity was reviewed in a report by the Toxicity Working Party of the Institution of Chemical Engineers (I.Chem.E.) [16]. The working party concluded that for active healthy people a 50% mortality level might be associated with a 30 minute exposure at 400 ppm. They were unable to recommend values for lower percentage mortality levels. Referring to Fig. 1 it is easily seen that the I.Chem.E. recommendation corresponds very closely to the 50% line from the Rijnmond Report industrial comments equation, whilst the view from Purdy et al. is that a 400 ppm concentration would produce 50% mortality for a duration of only 5 minutes.

We may gain further insight into the significance of the difference between these two views of chlorine toxicity by means of the following argument, in which the two sets of parameters are distinguished by the attachment of a subscript zero to one set. Taking eqn. (1) as the starting point, we rearrange this as

$$C^n t = \exp\left[\frac{P-a}{b}\right] \tag{4}$$

in which we refer to the quantity $C^n t$ as the exposure factor. For two different views of toxicity such as expressed in eqns. (2) and (3) the ratio of the exposure factors associated with a specified percentage mortality (and therefore a specified probit value) is

$$C^n t / C_0^n t_0 = \exp\left[\frac{P-a}{b} - \frac{P-a_0}{b_0}\right] \tag{5}$$

We may then fix the concentrations ($C=C_0$) or the times ($t=t_0$) as equal and ask what corresponding ratios of times or concentrations, respectively, yield the same percentage mortality from the two probit expressions. For equal concentrations the ratios of times is given by

$$R_t = t/t_0 = \exp\left[\frac{P-a}{b} - \frac{P-a_0}{b_0}\right] \tag{6}$$

The equivalent expression for the corresponding ratio of concentrations, R_c, for fixed times ($t=t_0$) is simply

$$R_c = (R_t)^{1/n} \tag{7}$$

The ratios R_c and R_t are plotted in Fig. 2, taking the values $a=-11.4$, $b=0.82$

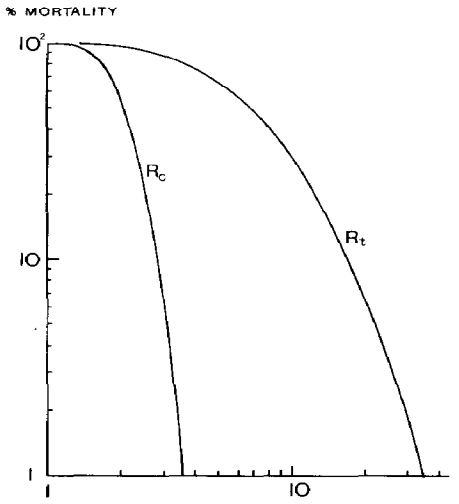

Fig. 2. Values of the concentration and time ratios R_t (eqn. 6) and R_c (eqn. 7) versus percentage mortality.

from the Rijnmond Report expression (eqn. 2) and $a_0 = -4.4$, $b_0 = 0.52$ from Purdy et al.'s expression (eqn. 3), with $n = 2.75$ in both cases.

Figure 2 shows quite clearly that the difference between these two probit expressions is more marked at lower levels of percentage mortality. In order to place these differences in context, one may consider the implications for emergency response by supposing, by way of illustration, that an emergency plan had a policy basis requiring that intervention for a reference accident should be achieved so as to limit the exposure to levels not exceeding 5% mortality. In practical terms this might be accomplished, for example, by ensuring effective termination of a release by closure of isolation valves within a specified time. Figure 2 shows that at this level the necessary intervention would have to be achieved approximately 22 times more rapidly under the assumption of the Purdy et al. probit expression than under the Rijnmond Report Industrial Comments probit expression. To illustrate this more vividly, if an intervention schedule were designed on the basis of the above stated policy, then a 10-minute completion time under the Purdy et al. expression would be equivalent to 3 hours 40 minutes under the Rijnmond expression. Such a difference evidently has substantial implications for resource allocation (e.g. staffing levels) and the setting of priorities in emergency response planning, and illustrates the practical need to improve our understanding of chlorine toxicity.

Deductions from modelled reconstructions of releases

Atmospheric dispersion models suitable for use with denser-than-air releases began to be developed in the 1970's, since when there have been several published accounts of attempts to reconstruct the reported effects of particular documented releases of chlorine, as well as other major hazard gases. Nussey et al. [17] used the dense gas dispersion models DENZ [18] and CRUNCH [19] for reconstructions of several 1st World War gas attacks in which chlorine was used, and for an accidental release that occurred in Norway in 1940. Withers and Lees [20] subsequently carried out a similar exercise for three 1st World War chlorine gas attacks, including one of the cases used by Nussey et al. (at Wulverghem, 30 April 1916). However, Withers and Lees chose to use a passive tracer model for atmospheric dispersion, thus making no allowance for the effects of density on the dispersion behaviour. Griffiths and Fryer [21] re-examined two of the attacks discussed by Withers and Lees, and presented dispersion estimates modelled both with and without allowance for density effects. They discussed the implications of the very different exposure patterns estimated under these two sets of assumptions.

Nussey et al. concluded that the chlorine toxicity criteria attributable to Dicken [22] yielded estimates of effects that were consistent with historical experience when used in conjunction with the dispersion models DENZ and CRUNCH. Withers and Lees concluded that their assessment supported the va-

lidity of a probit expression for chlorine toxicity that they had advanced in an earlier paper [23], which appears to be generally less stringent than the Dicken toxicity criteria, although a direct comparison requires some degree of interpretation, since the Dicken curves do not specify the associated percentage mortality in the way that a probit expression does. It should be noted that the conclusions of Nussey et al. [17] are neither supported nor challenged by Withers and Lees [20], who do not discuss the findings of the earlier paper. Griffiths and Fryer [21] concluded that the reconstructions of Withers and Lees did not yield robust conclusions concerning chlorine toxicity, a particular weakness being the use of an inappropriate dispersion model. However, even given the use of an appropriate model, there are numerous factors that contribute to the difficulty of obtaining reliable conclusions from such reconstructions, including the following:

- Available dispersion models provide concentration estimates that represent the ensemble mean behaviour of numerous single realisations of the release under given weather conditions. The variation of a single realisation about this mean is expected to be substantial, so that the parameters of a single incident can be expected to display substantial departures from the predicted mean behaviour.
- The information available from past incidents is often insufficient to permit an adequate description of the release conditions and the resultant toxic effects, and in particular to provide the required input data for dispersion modelling. This is often the case even for accidental releases that have been specifically investigated very soon after the event; it is especially true of the 1st World War gas attacks, for which information is often fragmentary or contradictory on such matters as the location, numbers and types of casualty, whether gas masks were in use, the rates, durations and total quantities of gas releases, and even the type of gas used.

The difficulties encountered in reconstruction thus centre upon the need for reliable information and estimation methods for (i) specifying the release, (ii) estimating the exposure, and (iii) specifying the incidence of harmful effects. Considering (i) and (iii), the information available has been identified and thoroughly assessed for the 1st World War gas attacks analysed in [17] and [20], and in related reviews [23, 24]. It can hardly be expected that any new source will now be found to enable that information be significantly improved. The use of dispersion models to estimate the exposure means that the exercise essentially involves the joint validation of the dispersion model and the toxicity relationship, as recognised by Nussey et al. Consistency with historical experience might thus be obtained fortuitously by the use, for example, of a dispersion model that underestimates concentrations in combination with a toxicity relationship that overestimates the response to a given exposure. Under these circumstances it is not realistic to hope to disentangle the relative contributions of the dispersion modelling and the toxic response uncertainties,

so as to validate both. Clearly, there is a requirement for an independent means of estimating exposure, so as to provide an intermediate reference point relevant to the validity of both the dispersion modelling and the toxicity relationship. It is with this need in mind that the investigations of vegetation damage reported here have been conducted. Apart from the establishment of an independent reference point, a further advantage of examining vegetation damage is that the pattern of exposure that might be established by post-incident investigation would relate to the single realisation of the actual release, rather than the ensemble behaviour predicted by dispersion modelling.

Review of earlier work

The damaging effects of chlorine on plant tissue have been noted in several reports of accidental releases [25–29], as well as in the reported effects of the 1st World War gas attacks (e.g. Table II in [17]).

There is a very large body of literature on the effects of normal-operation air pollutants on plants, with many fumigation experiments having been conducted on a variety of plant species. However, given this context of interest it is perhaps not surprising to find that there are only a few papers dealing with the effects of chlorine, which is not one of the major air pollutants. Furthermore the exposure periods of interest in air pollution studies are very long compared with the likely durations of accidental exposures. We have found only a small number of papers of direct relevance to this situation, and in the following paragraphs we have selected a few of these to illustrate the main points. The findings of other papers not listed here are in keeping with the indications of those selected.

Considering first the literature on accidental releases, Stout [25] described the nature of chlorine damage to lettuce, weeds and grasses observed some days after an accidental release from a sewage treatment works. Lettuce developed necrotic lesions along the margins of the outer leaves, extending inwards in solid patches in severe cases. Less severe damage was in the form of brown spots on the leaf upper surface. Grasses and weeds showed pale yellow/white bleached areas at the leaf tips. Hindawi [26] referred briefly to an accidental release which caused severe damage to trees, shrubs and ornamental plants. Leaves developed marginal necrosis and necrotic spots. On the day after the release leaf-fall was observed on tomato plants, trees and especially on privet hedges. Brennan et al. [27] surveyed vegetation at 5 days and 14 days after an accidental release of chlorine from a sewage treatment plant. Widespread vegetation damage was observed out to 1500 feet from the point of release. Flowers, trees and weeds were examined. Bleaching, interveinal necrotic spots and marginal burning of the leaves were all common, and in rarer cases complete necrosis of the leaf. There was no evidence of defoliation, and healthy new growth had returned on most plants after 2 weeks.

Harger [28] carried out a very detailed survey of vegetation damage one month after a release from a chlor-alkali plant. Numerous species showed substantial damage at distances out to 1 mile from the release point. The incidence of damage was recorded as the proportion of leaves showing damage on sample sections of the plant species. Most species within 1 mile of the release showed 30% to 90% incidence of damage. Although the survey was extensive and quantitative, information necessary to estimate the exposure conditions was not available; estimates of the quantity of chlorine released ranged from 2 to 30 tons.

The earliest study of which we are aware that attempted to relate plant damage symptoms to known exposures after an accident was reported in 1979 by Booij [29], on an accidental release stated to have occurred some years earlier, but otherwise not identified. It was estimated that about 3.6 tons of chlorine vapour was released in about 15 minutes, evolving from a total release of 5 tons inside a building. Vegetation damage in the vicinity was widespread, and damaged nettle plants were photographed within 72 hours of the accident. Unaffected nettles from the area were then exposed to known concentrations for known durations, and these nettles were photographed, this time a few hours after exposure. The photographs were ordered according to severity as judged by a panel. Booij concluded that the effect was proportional to a combination of concentration C and duration t of the form C^2t. Unfortunately the details of the investigation are reported only in qualitative terms. The criteria for the judgement of severity of visual damage are not specified, and the two sets of photographs were taken at different intervals from the exposure, so that there is no allowance made for the effect of symptom development with time.

Turning to fumigation experiments, Thornton and Setterstrom [30] exposed tomato, buckwheat and tobacco to chlorine in concentrations between 1 and 1000 ppm for durations of between 1 and 960 minutes. The time required to produce injury to 50% of the surface area (LT_{50}) when exposed to 1000 ppm was 0.8, <4 and 0.5 minutes for leaves and 22, 120 and 60 minutes for the stems of tomato, buckwheat and tobacco respectively. Lower concentrations of gas increased the LT_{50} time. Greater injury appeared during clear rather than cloudy weather.

Zimmerman [31] exposed 19 species of plants to chlorine at concentrations ranging from 0.46 to 4.67 ppm for durations of 20 to 480 minutes. Sixteen species were found to be susceptible. Injury appeared first on the middle-aged leaves, followed by the oldest and then the youngest leaves, and was similar to that caused by sulphur dioxide. The plant material took on a cooked appearance and finally turned a straw/brown colour depending upon species. Medium-to-considerable injury was associated with leaf fall. Wilted plants showed increased resistance to damage compared with turgid plants.

Benedict and Breen [32] selected weeds representative of those found in most climatic areas in the United States. They exposed plants of two different

ages to 0.5 and 2.5 ppm chlorine under high and low soil moisture conditions for 4 hours. Broad leaved species developed interveinal necrotic areas whilst grasses developed marginal streaks which progressed to the main vein in the region between the tip and the point where the leaf bends. The middle-aged leaves on each plant were more severely affected, although 3-week and 6-week old plants were equally affected. Low soil-moisture levels were associated with decreased sensitivity to chlorine.

Brennan et al. [33] investigated the effects of lower chlorine concentrations on many plant species to enable the recognition of episodes of chlorine release in nature. Concentrations of 0.1–1.5 ppm for 4 hours produced a variety of symptoms. Water soaking of foliage and wilting was followed by the development of bleaching and necrosis, the position and colour of necrotic tissue varying between species. Younger leaves were more resistant to injury. An examination of the effects of moisture upon the leaves of tomato plants showed no difference in response whether the leaves were wet or dry. Periods of darkness following fumigation decreased damage, but darkness prior to fumigation had no effect. Low soil moisture levels were associated with decreased incidence of injury, confirming the observations of Benedict and Breen.

Basis of the present study

The investigations reported here extend earlier work conducted from 1980 to 1984 with sponsorship from the Health and Safety Executive. Following a preliminary feasibility study [34], the main investigations were carried out by Lydiard [35], who established the techniques and methods of interpretation that have been used here. In an extensive investigation based on fumigations of different plant species with high concentrations either of chlorine or ammonia in air, Lydiard examined numerous types of effect, including visual damage to the plant tissue, changes in plant enzyme activity and in leaf chlorophyll content, weight changes in the leaf tissue, changes in the ion content of plant tissue and soil, and effect on soil ammonification and nitrification processes. The main groups of experiments were carried out on *Lolium perenne S.23* (perennial ryegrass) and *Trifolium repens c.v. Huia* (white clover). Chlorine fumigations covered the concentration range 35 to 710 ppm for ryegrass, and 35 to 157 ppm for white clover, at durations of 5, 10 or 20 minutes. The ammonia fumigations similarly covered the range 100 to 800 ppm, at 5, 10, 20 or 30 minutes duration. Lydiard concluded that the incidence of visual damage to leaf tissue provided a means whereby exposure characteristics could be quantitatively related to observable effects. The particular characteristics suitable for quantification were identified as: (i) the percentage number of leaves showing necrotic damage within specified severity ranges, these being defined as the percentage of the individual leaf area affected in the ranges 1–10%, 11–25%, 26–50%, 51–75% and 76–100%; (ii) the total percentage number of leaves

damaged; and (iii) the time taken for the symptom incidence to develop to a maximum (either as total percentage incidence or within a given severity range). It should be noted that these percentages relate to the total leaf count on the fumigated set of plants at the time of counting, which usually extended to about a week in order to encompass the time taken for the maximum incidence of damage to develop. During this survey period the total leaf count usually changed, due both to leaf drop and to the appearance of new growth (the latter most often dominated, yielding a net increase). Whilst it may seem unsatisfactory thereby to include unfumigated leaves in the overall count, it was found to be very difficult to devise a scheme that would achieve the desired segregation, but which would not seriously encumber the application of the method in a real post-accident investigation, where the difficulties encountered would be considerably greater than in the laboratory situation. It was judged that the method was more likely to be successful for field use if it was based on a recognition of the practicalities of such investigations. Accordingly, the strategy described above was adopted on the grounds that practicability in the field was a crucial requirement.

Lydiard found that the two species showed a similar sensitivity to exposure to ammonia, and identified three regimes of response; (i) less than 100 ppm concentration, which produced no necrosis, (ii) 100 to 400 ppm in which the maximum incidence of visual damage in the 76 to 100% range provided discrimination between concentrations, and (iii) above 400 ppm, which produced effectively 100% incidence in the 76 to 100% severity range. Discrimination on the basis of duration of exposure was available in regime (ii) only at the lower concentrations. However, in the case of the chlorine fumigations, whilst a similar pattern was revealed, white clover was found to be much more sensitive than perennial ryegrass.

For ryegrass the regime (ii) above was found to extend at least from 35 to 710 ppm. As illustrated in Figure 3, results of 10-minute fumigations at these concentrations showed maximum incidence of damage in the 76 to 100% severity range of 46% at 35 ppm, increasing at a near-linear rate to 87% at 710 ppm. By contrast, the total incidence (at all severity ranges) was 83% at 35 ppm, rising rapidly to effectively 100% incidence at 100 ppm and all higher concentrations, and therefore providing a much less useful graduation of symptom incidence with exposure.

For the same 10-minute exposures white clover showed maximum incidence values in the 76 to 100% severity range of 66% at 35 ppm, 86% at 100 ppm, and 90% at 157 ppm.

The programmed end of the experimental phase of the project imposed a limit on the extent of the chlorine fumigations, preventing effective investigation of the no-necrosis regime and the 100% incidence-in-the-top-severity-range regime that was a feature of the ammonia fumigation results described earlier (regimes (i) and (iii) respectively). The investigations reported here

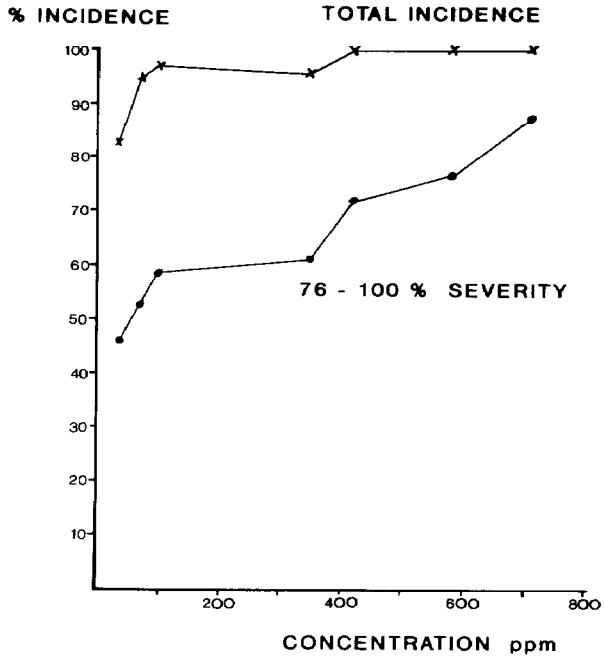

Fig. 3. Observed maximum incidence of leaf damage to *Lolium perenne S.23* following 10 minute fumigations with chlorine at various concentrations, from Lydiard [35].

are based on M.Sc. dissertation work by Smith [36], and have been carried out in order to extend the work of Lydiard in respect of determining the characteristics of regime (i) for white clover. Although the need for investigation applies just as well for perennial ryegrass, white clover has been given priority for two reasons; firstly, its greater sensitivity should enable useful post-accident investigations to extend to greater dispersion distances (i.e. lower levels of exposure) than for ryegrass, and secondly, the leaf shape permits a more definite and easier estimate of the area of leaf damage to be made than is the case for the elongated leaves of ryegrass.

Experimental method

The fumigation chamber used in these experiments was one of a pair used by Lydiard, consisting of a rectangular perspex box of dimensions $0.7 \times 0.5 \times 0.4$ m (volume 140 l) with a sealable lid covering a hole of dimensions 0.2×0.3 m cut into the top. Gas inlet and outlet ports of 25 mm diameter were located one in the top next to the lid, and the other at the bottom of one of the end walls. Lydiard adopted two distinct arrangements to achieve the desired fumigation concentrations. The first involved supplying the chamber with air at a volume

flow rate of about 120 l min^{-1}. This air supply was fed first through a charcoal filter, and then via a flowmeter and a total-volume gas meter to the chamber. The contaminant gas was introduced at a measured flow rate via a side branch into the main air pipe. This once-through flow arrangement ensured that the carbon dioxide/oxygen balance in the chamber was not altered by photosynthesis during the experiment. This method proved unsatisfactory where low concentrations of gas (less than 100 ppm) were required, because of the performance of the rotameter type gas flowmeters at the lower end of the flowrate scale. Chlorine concentrations were determined not only from flow measurements, but also by bubbling the air–chlorine mixture through a 2% acidified potassium iodide solution, and titrating with 0.1 *M* sodium thiosulphate using a starch indicator. The second method involved sealing the gas inlet and outlet ports and injecting a known volume of pure chlorine gas via a resealing membrane and keeping the chamber well stirred with a small battery driven fan. This method was judged to be satisfactory for short duration exposures (10 minutes or less), provided the amount of plant material in the chamber was small, so that the photosynthesis effect on carbon dioxide/oxygen balance was not excessive. The second method was adopted in the experiments reported here, the titration involved being carried out by bubbling 6 l of chamber air through 100 ml of 0.2% acidified potassium iodide solution, and then titrating against 0.001 or 0.01 *N* sodium thiosulphate solution using a starch indicator. Additionally, the chlorine concentration in the chamber was measured directly every 30 s using a Sabre-Interscan series 4000S portable chlorine monitor, the gas being drawn to the detector via one of the chamber gas ports, and the return being made via the other. The monitor was an electrochemical voltametric device in which the current required to achieve electrochemical reduction of a proportion of the contaminant gas is used as a measure of the gas concentration. This particular model gave a directly displayed reading in ppm, and was calibrated by the suppliers immediately prior to use in these experiments.

White clover seeds that were genetically identical to those used by Lydiard (*Trifolium repens c.v. Huia*) were sown in seedtrays in John Innes potting compost. These were subsequently thinned out so that at the time of fumigation, when the plants were between 10 and 16 weeks old, each seed tray contained between 150 and 300 leaves (it should be noted that each leaf of white clover consists of three leaflets; the whole leaf is the basis for the interpretation of percentage leaf area damaged).

In addition to these sown plants, turves of mixed species and genetic composition containing *T. repens* were obtained from an undisturbed patch of ground at the Jodrell Bank field station of the Department of Botany (University of Manchester). The fumigation apparatus and the plants were kept together on a roof-top location in central Manchester during the experimental programme which was carried out over the period July–August 1988. A sequence of 16 fumigations was completed, exposing about 2750 leaves in all.

TABLE 1

Summary of chlorine fumigations on white clover; exposures are all of 10 minutes duration, and on *T. Repens c.v. Huia* except for the two turf experiments (15 and 16)

Experiment No.	Initial concentration C_0 (ppm)	Mean-to-initial concentration ratio, C_{mi}	Exposure conditions
1	100	[a]	Bright
2	70	[a]	Bright
3	35	0.31	Bright
4	30	0.42	Bright
5	25	0.49	Bright
6	20	0.33	Bright
7	15	0.32	Bright
8	10	0.38	Bright
9	5	0.38	Bright
10	1	—[b]	Bright
11	20	0.67	Bright; wilted leaves
12	20	0.4	Bright; wet leaves
13	20	0.45	Cloudy
14	20	0.55	Dark
15	20	0.27	Bright; turf
16	5	0.46	Bright; turf

[a] Data not available for these cases.
[b] Insufficient meter resolution to obtain reading beyond 3 minutes.

Fumigations were all of 10 minutes duration, with concentrations in the range 1 to 100 ppm, but with the majority in the 1 to 35 ppm range. Table 1 summarises the conditions of the 16 experiments, most of which were conducted in bright sunny weather. In order to examine the influence of other conditions, some fumigations were carried out under cloudy conditions, or in the dark, and with plants that were wilted or with leaves made wet by spraying.

Following exposure to chlorine the plants were kept in the open and observations were made daily for up to 7 days. Damage was recorded in three ways. Firstly, the percentage number of leaves showing necrosis in the severity ranges previously specified was recorded, together with the total incidence in all severity ranges. These measures were recorded daily to reveal the time taken for symptoms to reach their maximum incidence. A qualitative description of weather conditions was recorded for each day of this symptom development period. Secondly, a descriptive record of visual damage characteristics was made, recording the type of marking, the age of the foliage affected, the distribution of symptoms on the leaf, whether one or both leaf surfaces were affected, and the distribution of symptoms over the entire plant. Thirdly, photographs were taken to supplement the other records. Observations were also made on unex-

posed control plants, so that comparisons could be made against any background level of leaf necrosis from other causes. Such necrotic leaves are normally present in small numbers; where present, they were not removed from the plants prior to fumigation.

Results

Table 2 provides a summary of the observed damage characteristics. Figure 4a shows an undamaged leaf, whilst various degrees and distributions of damage are shown in Figs. 4b to d. The results of these experiments are extensive, and are reported fully in Smith [36]. Here we present and discuss a limited selection of results which illustrate the main points relevant to the particular context of this paper.

Before entering discussion of the results on leaf damage it is necessary to clarify the interpretation of the concentrations quoted in each experiment. The concentration specified is that measured by the electrochemical chlorine monitor at the beginning of each experiment. The measurements taken at 30-second intervals throughout each experiment showed that the concentration in the chamber fell steadily over the 10 minutes of the fumigation. In addition to measurements made during fumigations of plants, other measurements were made with soil only in the chamber to investigate the time-dependent behaviour of concentration. With soil only in the chamber the pattern was a rapid

TABLE 2

Description of damage seen on plant tissue during the experiments

Damage observation	Effect of chlorine
Type of Marking	Extensive bleaching of leaves appeared within 24 hours at 70–100 ppm. At lower concentrations bleaching of margins only. Yellow/brown necrotic tissue begins to develop 24–48 hours after fumigation. 'Cooked' appearance of leaves precedes necrotic tissue development at 10–35 ppm Wilting within 10 minutes of fumigation at 15–100 ppm
Age of foliage affected	Older and middle-aged leaves. Leaves not unfurled at time of fumigation are unaffected.
Distribution of symptoms on the leaf	Severely affected leaves have whole surface covered. Other leaves show damage avoiding the main veins.
Surface of the leaf affected	Both surfaces, except in dark exposure and 'bright' exposure with wilted leaves where upper surfaces only were affected.
Distribution of symptoms over the entire plant	Leaves most severely affected, stems rarely affected, flower heads unaffected.

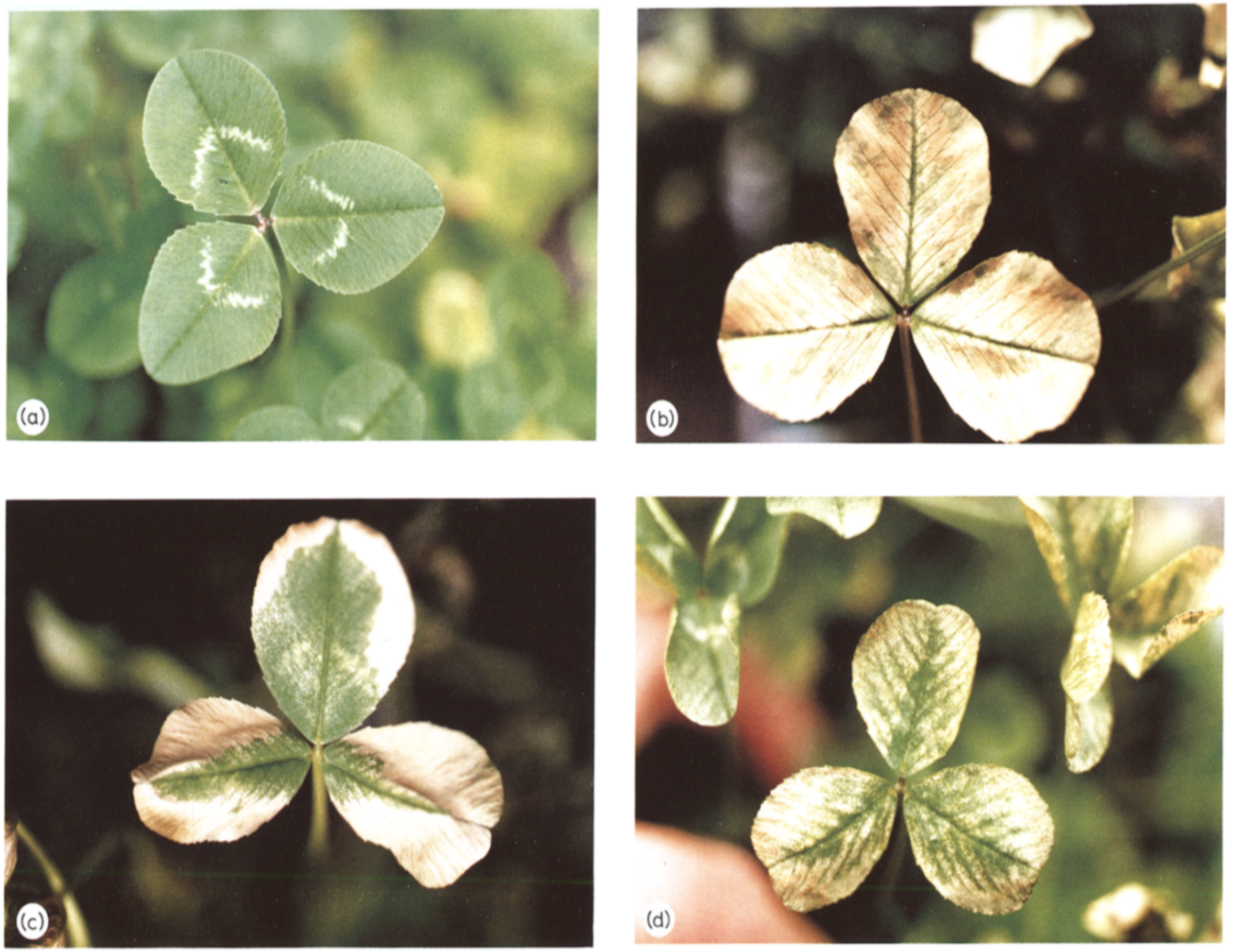

Fig. 4. Chlorine damage to white clover: (a) undamaged leaf; (b) 76–100% severity range (note the avoidance of the main veins); (c) 51–75% severity range: damage is confined to the leaf margins; and (d) showing diffuse damage in the 51–75% severity range.

fall to about 90% of the initial concentration in the first 30 s, followed by a linear decline to about 60% of the initial concentration at the end of the 10 minutes. With the sown clover plants in the chamber the concentration fell at a rate that declined as the concentration became smaller, suggesting an approximately exponential decay. The reduction in concentration in these cases is clearly due principally to uptake by the plants, which varied according to conditions in a systematic way that was consistent with the observations of damage. For example, plants fumigated in a wilted condition showed less damage and depleted the chlorine concentration to a lesser degree than plants that were in a turgid (i.e. unwilted) state. This is related to the degree of stomatal opening, which would encourage chlorine uptake into the leaf in the turgid state. As noted above, concentrations were measured every 30 s, so that the concentration–time profile is known for each experiment. However, we have found it useful to summarise these data by relating the mean concentration over the 10-minute exposure, $\bar{C}$, to the initial concentration, C_0, and specifying the mean-to-initial concentration ratio C_{mi} as a parameter, as listed in Table 1.

The behaviour of C_{mi} for a set of experiments that were nominally identical except for the value of C_0 is evident in experiments 3 to 9 in Table 1 (experiment 10 is in the same group but the concentration was below the instrument resolution beyond 3 min). For this group the mean value of C_{mi} is 0.376, with a standard deviation of 0.06. The least uptake indicated is for soil only, for which C_{mi} is 0.73. It should be noted that the turves contained a much greater loading of foliage than the sown trays of white clover, since the turves contained many species closely packed and were not thinned to be equivalent to the sown trays. Results in terms of the total and the top severity range incidence measures described earlier are displayed in Figs. 5 to 11.

Results from experiments under bright conditions on sown white clover are shown in Fig. 5 (experiments 1 and 2), Fig. 6 (experiments 3, 4 and 10) and Fig. 7 (experiments 1 to 10). For the 100 ppm and 70 ppm fumigations the maximum incidence occurred by the end of day 1. The incidence was high in both total and top severity range measures, and although the lower incidence is associated with the lower concentration, the difference is small and not sufficient to enable reliable discrimination between concentrations. The total incidence in the control group is shown in Fig. 5. Figure 6 displays results for 35, 30 and 1 ppm. At 35 and 30 ppm the development of maximum symptom incidence is at day 3 for the top severity range, and at day 2 (30 ppm) and day 1 (35 ppm) for total incidence. This trend towards longer times to the maximum incidence for lower concentrations was a consistent feature of the results. The total incidence for the 1 ppm fumigation is indistinguishable from that in the unfumigated control group shown in Fig. 5. The discrimination on the basis of concentration shown in Fig. 6 is evident in the total incidence, but is much stronger in the top severity range incidence. Figure 7 summarises the results from experiments 1 to 10, showing the maximum incidence observed in both

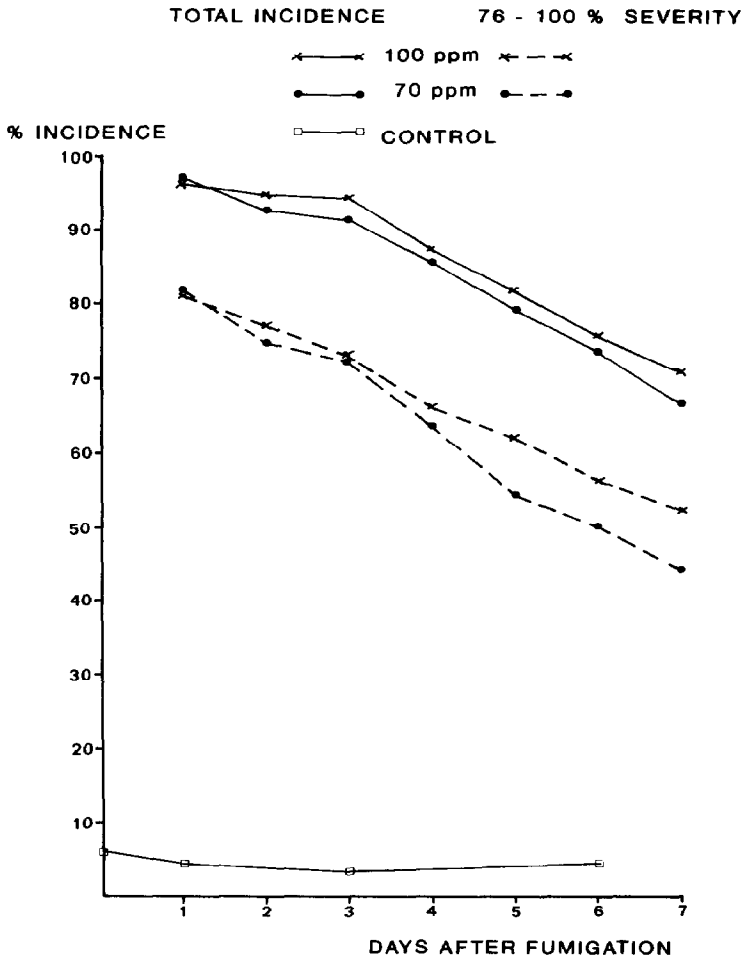

Fig. 5. Observed development with time of the incidence of chlorine damage to leaves of white clover for experiments 1 and 2 (see Table 1).

the total and the top severity range categories. In addition to these composite results, the following observations were noted. At chlorine concentrations between 15 and 100 ppm wilting of leaves began within 10 min of completion of the fumigations. Below this concentration no wilting was observed. The type of leaf marking varied with concentration. At 70 and 100 ppm gross damage appeared within 24 hours of exposure covering almost all the leaf area with a papery, bleached and yellow/brown necrotic tissue which caused the leaves to curl inwards. Both upper and lower leaf surfaces, as well as a few stems were affected. At concentrations between 10 and 35 ppm, curling of the leaves was a persistent feature, although the bleaching described above was less severe. It tended to occur at the leaf margins whilst a dark brown/yellow necrotic tissue

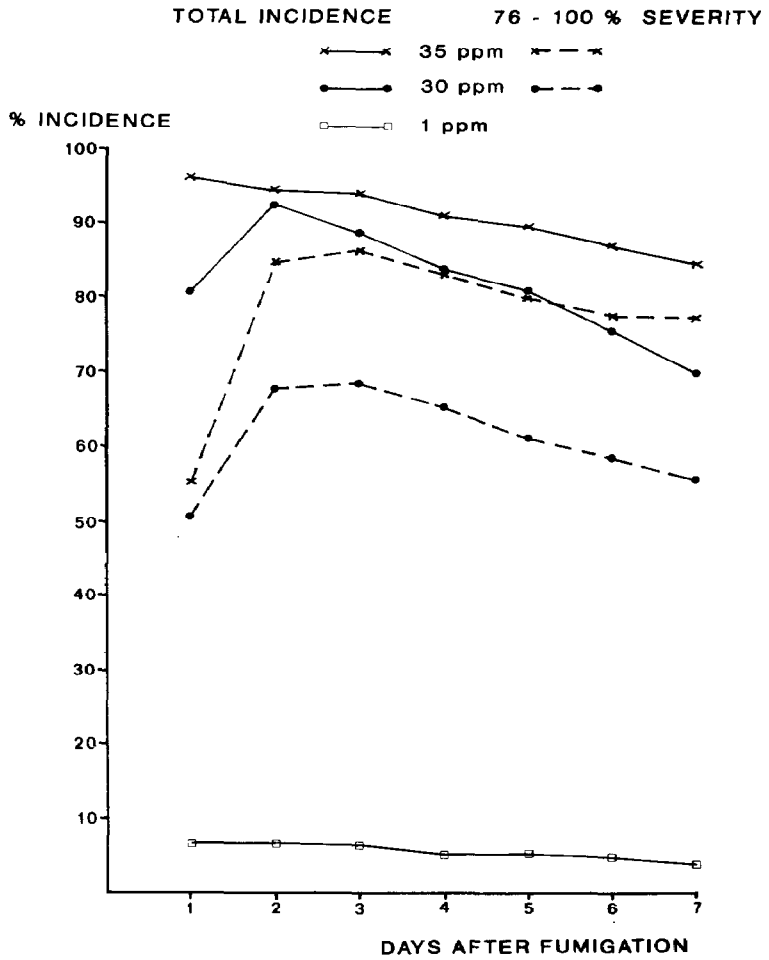

Fig. 6. Observed development with time of the incidence of chlorine damage to leaves of white clover for experiments 3, 4 and 10 (see Table 1).

spread over the interveinal leaf areas. Many leaves took on a dark green 'cooked' appearance within 24 hours of exposure which was replaced with necrotic tissue in subsequent days. Both leaf surfaces were affected, but stems and flower heads were not. Necrotic leaves were papery to the touch. As chlorine concentrations were reduced the 'cooked' appearance became a more important feature of early observations and necrotic tissue development was less extensive, becoming more diffuse and avoiding the major leaf veins. At 5 ppm most leaves maintained a normal appearance. Those that were affected showed only slight curling and small area of necrotic tissue.

The incidence of damage in the top severity range proved consistently to be

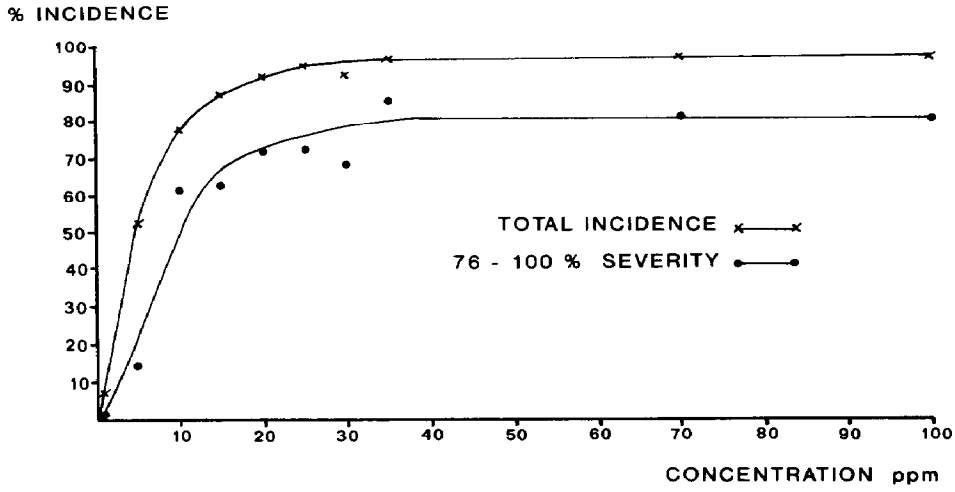

Fig. 7. Observed maximum incidence of chlorine damage to leaves of white clover versus concentration for experiments 1 to 10 (see Table 1).

of most use in assessing the response. The incidence of damage in the other severity ranges was low, typically a few percent, and never more than about 15%, even for high values in the top severity range. As shown in Fig. 7, the maximum total incidence of damage was in excess of 90% for concentrations of 20 ppm and above. Below 20 ppm the maximum total incidence of damage gradually falls to about 80% at 10 ppm and falls sharply thereafter until it shows no significant difference from that of the control at 1 ppm (significance was calculated at the 95% level by using the chi-square test). However, there is still a large amount of damage (51.9% maximum) at 5 ppm. Similarly, the maximum damage in the 76–100% severity range is in excess of 80% for exposures to concentrations of 35 ppm and above. This figure falls to about 62% at 10 ppm, and then sharply declines to 0.6% at 1 ppm. The reduction in total damage with decreasing concentration is associated with a declining proportion of damage in the top severity range. At 5 ppm damage is divided more or less evenly between the severity categories. At 10 ppm and above most damage is in the 76–100% range.

Figure 8 shows results from experiments 6, 13 and 14, under bright, cloudy and dark conditions respectively. Exposure under bright conditions gave the highest total incidence, whilst cloudy and dark exposures resulted in very similar total incidence values at a level lower than that for the bright conditions. This similarity in the cloudy and dark cases is at first surprising, since the closure of the leaf stomata in the dark would be expected to reduce chlorine uptake. However, when the incidence of damage within the 76–100% severity range is examined it can be seen that damage occurring in the dark is in fact less severe, being only 19.4% maximum compared with 48.1% under cloudy

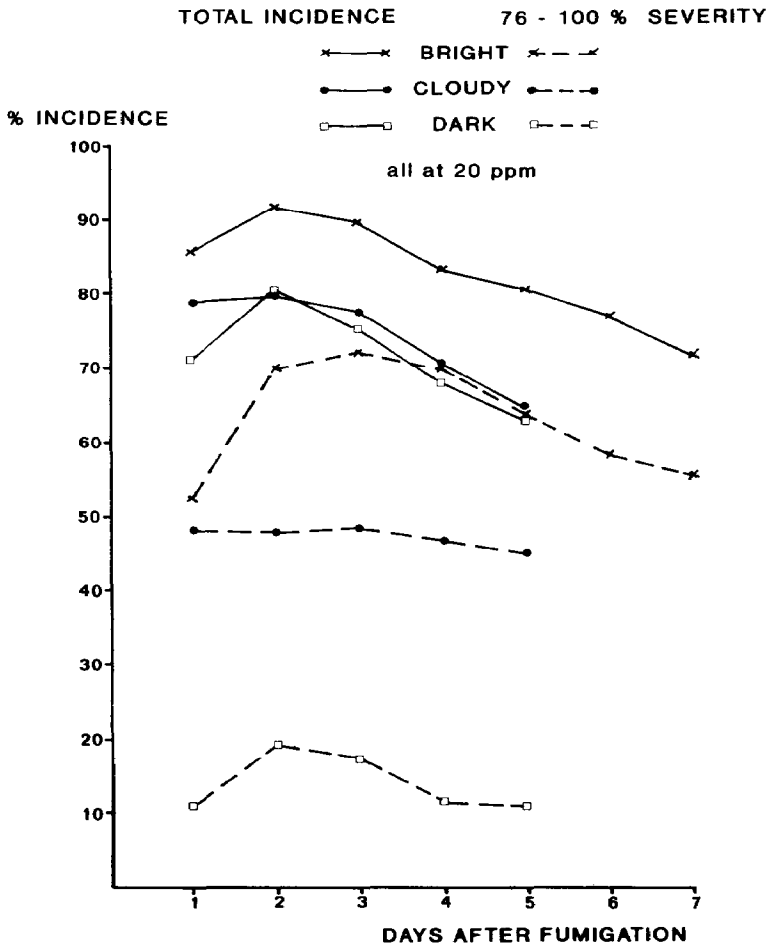

Fig. 8. Observed development with time of the incidence of chlorine damage to leaves of white clover for experiments 6, 13 and 14 (see Table 1).

conditions. Examination of data for the other severity ranges shows that for the dark exposure most damage occurred in the 1 to 10% range, whilst cloudy and bright exposures caused most damage in the 76–100% range. Damage may have occurred during dark exposures because chlorine had passed through the leaf epidermis or because the leaf stomata had not fully closed, even though the plants were kept in the dark for several hours prior to exposure.

Figure 9 shows results from experiments 6, 11 and 12, with dry leaves, wilted leaves and wet leaves respectively. The experiments were all carried out in bright conditions, with one dry and the wet leaf case being for turgid plants. The presence of moisture on the leaves had little effect on the maximum total

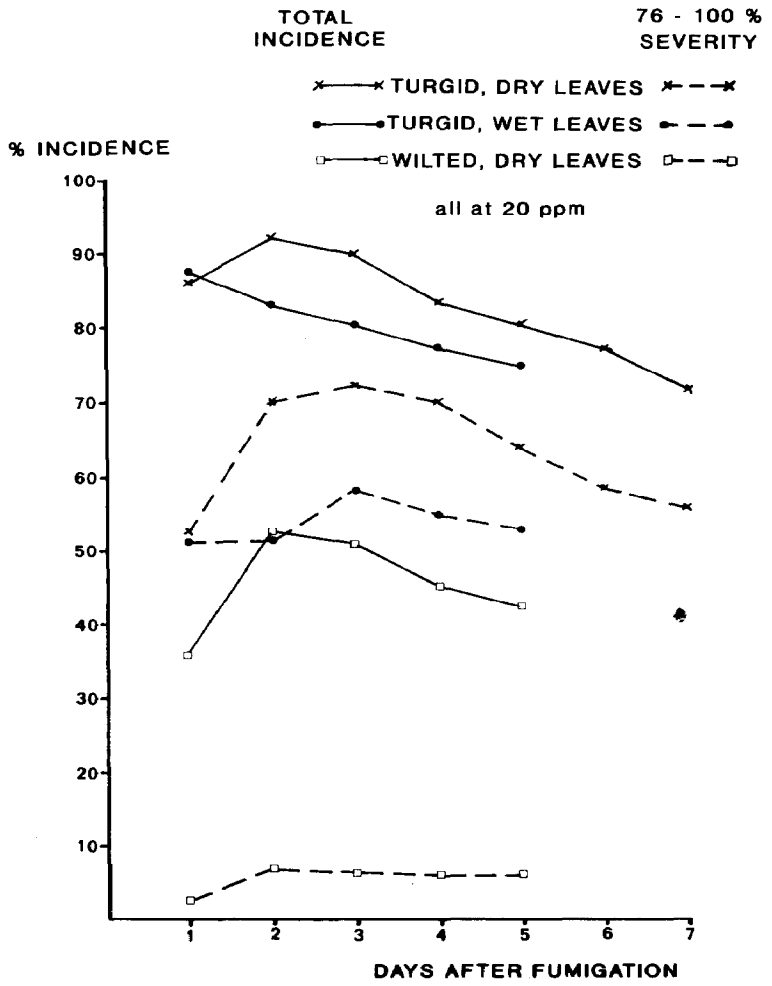

Fig. 9. Observed development with time of the incidence of chlorine damage to leaves of white clover for experiments 6, 11 and 12 (see Table 1).

incidence of damage produced, but a more substantial difference was seen in the top severity range, where the incidence was reduced for the wet leaves. There was, however, a marked reduction in damage if the leaves were wilted, the maximum values being 52.5% (total incidence) and 6.8% (76–100% range), compared with 91.8% and 72.3% respectively for turgid dry leaves under the same conditions.

Results of experiments comparing the sown white clover and the white clover in the turves are presented in Fig. 10 (experiments 6 and 15) and Fig. 11 (experiments 9 and 16). Sown plants and turf plants were exposed to 20 ppm and 5 ppm under bright conditions. The figures show the turf plants to be less

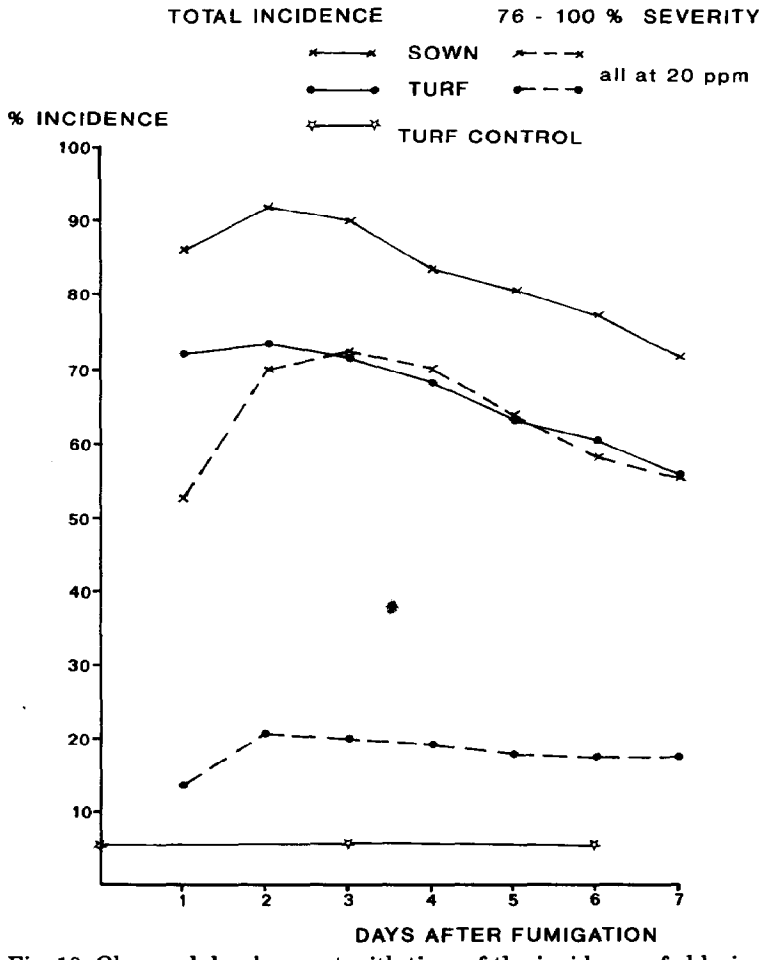

Fig. 10. Observed development with time of the incidence of chlorine damage to leaves of white clover for experiments 6 and 15 (see Table 1).

prone to damage than the sown plants. At 20 ppm there is a large difference in the maximum total damage between the sown material (91.8%) and turf material (73.6%), and an even larger difference in the maximum incidence of damage in the 76–100% category which shows 72.3% and 20.9% respectively. At 5 ppm the maximum total incidence was similar at 51.9% (sown) and 55.5% (turf), whereas the results for maximum incidence in the 76–100% category were 14.2% and 4.3%. In interpreting this difference in response it should be noted that the turf plants were different from the sown plants not only in their genetic composition, but also in soil type, and in the foliar loading, since the turves contained other species in addition to the clover.

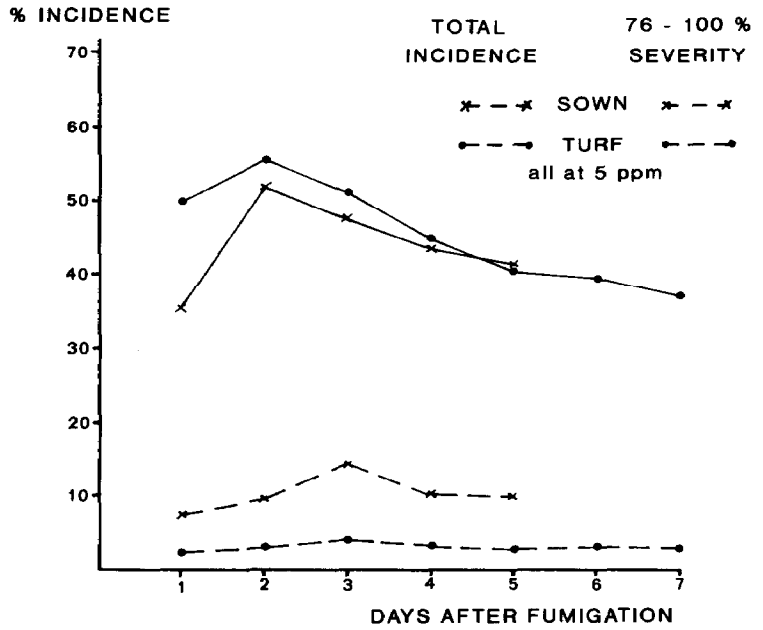

Fig. 11. Observed development with time of the incidence of chlorine damage to leaves of white clover for experiments 9 and 16 (see Table 1).

Comparison with results from Lydiard

Comparison between our results and those of Lydiard for the same species of white clover can be made for the 35, 70 and 100 ppm fumigations, although there were differences in experimental method. Lydiard used the once-through maintained-flow method of fumigation for the 100 ppm case, whereas we used the single injection method for all cases, which was also used by Lydiard for the 35 and 70 ppm cases. We have already described the concentration reduction over 10 min measured in our experiments, which would also have affected Lydiard's 35 and 70 ppm cases. However, Lydiard relied on the initial chlorine concentration for the single injection method being nominally that defined by the known quantity injected mixed into the 140 l of the chamber with no losses, which we have found would be an overestimate. This difference is probably less important than the change in concentration over the subsequent 10 minutes imposed by different chlorine uptake rates in different experiments, which we have characterized in terms of the mean-to-initial concentration ratio developed from measured concentrations. Further differences in the two studies can be expected to arise because of the ages of the plants used. Lydiard's were 4 weeks old at fumigation, whereas ours were 10 to 16 weeks old, which may affect the sensitivity to chlorine damage. Bearing these points in mind, we have

compared the two sets of results and find that for the 70 and 100 ppm cases the values of maximum incidence (both total and in the top severity range) are within a few percent of each other in the two studies, the largest difference being 5%. However, at the 35 ppm level we find a consistently higher maximum incidence on both measures, the largest difference being 24% on the total incidence. Additionally, the time taken to maximum symptom development was longer in Lydiard's study, at 5 to 6 days, whereas our equivalent figure is 1 to 3 days.

Summary of experimental findings

Fumigations of *Trifolium repens c.v. Huia* (white clover) with chlorine for 10-minute exposures at various concentrations in the range 1 to 100 ppm reveal a region of progressive increase in visual leaf damage in the range 1 to 20 ppm. Segregation of symptoms into severity ranges based on the proportion of individual leaf area affected provides a better means of assessing response than does total percentage incidence of leaves damaged at all levels of severity. The severity range 76–100% leaf area damaged provides the most consistent method of interpreting damage, and of discriminating between related experimental factors. Environmental factors influenced the incidence of damage, which was greatest for turgid plants exposed under bright sunny weather conditions. Damage incidence was reduced for plants that were exposed in cloudy or dark conditions, or that were wilted due to lack of moisture prior to exposure. White clover of a different genetic strain, obtained from uncultivated turves, was broadly less susceptible than the sown samples. Chlorine concentration depletion in the fumigation chambers was substantial over the 10 minute exposure duration, the gas being admitted in a single injection at the beginning of each fumigation. Although constituting a limitation in one respect, the use of this method had the virtue of revealing differential chlorine uptake that was consistent with the incidence of damage, a feature that would not have been evident in a maintained flow system.

Use of vegetation damage assessment in accident investigation

The results reported here support the view that a useful element of accident investigation could be based on the interpretation of vegetation damage. White clover as investigated here shows a graduated response at low levels of concentration (1 to 20 ppm for the conditions of these tests) which is most readily interpreted in the 76–100% severity range based on individual leaf area affected, as described in the damage characterisation scheme used here. Interpretation based on the total incidence of damage provides a more limited range of assessment, with less ability to discriminate between exposure characteristics. However, counting the incidence in the 76–100% severity range is a more

involved task than counting the total, and in field use it may be appropriate to use both schemes, applying the 76–100% scheme only to a few selected locations.

The incidence of damage is related to a variety of environmental and species-dependent factors. Thus, interpretation of damage observed at a particular site would require that the appropriate locally occurring plant species be calibrated by conducting controlled fumigations on specimens growing in the vicinity but unaffected by the accident. The results could then be used to deduce the exposures suffered by the plants damaged in the accident. Sites with a recognised hazard might usefully be prepared by establishing plots of suitable species, such as white clover, in the vicinity; in the event of a release occurring an indicator with a known response pattern would then be available.

Clearly, the fullest use would have to be made of independent information on the duration of the accidental exposure, if it were available. Symptoms of the kind investigated here are typical of the effects of acute exposures, but they are not specific to the substance involved. Knowledge of the identity of the material released would be required independently. The calibration fumigations would need to be carried out very soon after the accidental release and in conditions as near as possible to those at the time of the release. Given the time-dependent character of symptom development as revealed in this and earlier studies, the site investigation of vegetation damage would need to start within a day of the actual release. Provided that resources could be made available to incorporate these requirements into the established procedures of the accident investigation, there seems to be every reason to expect that significantly useful results could be obtained. The studies reported here could usefully be extended to cover other plants species and other hazardous substances.

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