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ACUTE INHALATION TOXICITY OF CHLORINE IN RATS AND MICE: TIME–CONCENTRATION–MORTALITY RELATIONSHIPS AND EFFECTS ON RESPIRATION

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Summary

The acute inhalation toxicity of chlorine was studied in rats and mice using whole body exposure. Groups of five males and five females were exposed to different concentrations of chlorine for various periods of time and survivors were observed for 14 days thereafter. Satellite groups were sacrificed two days after exposure to obtain intermediate pathological information. Animals of the satellite groups were also used to investigate acute respiratory responses to exposure with chlorine.

For rats the relationship between any LC value, concentration, and time of exposure could be described by the following probit equation:

$$P = -16.67 + 1.33 \ln C - 4.31 \ln T + 1.01 \ln C \times \ln T$$

yielding the LC-50 values: 5-min LC-50 = 16.9 g/m^3, 10-min LC-50 = 5.6 g/m^3, 30-min LC-50 = 2.0 g/m^3, and 60-min LC-50 = 1.3 g/m^3, and LC-01: 5-min LC-01 = 7.3 g/m^3, 10-min LC-01 = 3.0 g/m^3, 30-min LC-01 = 1.2 g/m^3, and 60-min LC-01 = 0.8 g/m^3.

For mice the response relation could be described by:

$$P = -33.74 + 4.05 \ln C + 2.72 \ln T$$

yielding the LC-50 values: 10-min LC-50 = 3.0 g/m^3 and 30-min LC-50 = 1.5 g/m^3.

The breathing pattern and the minute volume in rats changed during exposure. Rapid shallow breathing directly after the start of exposure was followed by apneustic breathing. Near the end of exposure the breathing pattern changed again in a few animals in a manner suggestive of pulmonary oedema formation. The minute volume decreased to about 20% of the pre-exposure value. Incorrect adaptation of the ventilation — either too much or too little — increased the incidence of lethality.

In rats exposed to high concentrations of chlorine death occurred on the day of exposure and on the first and second day thereafter. Only one animal out of 56 deaths died in the second week of the observation period. In mice, 17 out of 59 deaths occurred in the second week of the observation period. Histopathological examination of the lung showed focal oedema and aggregates of polymorpho- or mononuclear inflamatory cells. Additionally, hyperplasia in the lining epithelium of the larynx and trachea was observed in animals killed at day 2. Only animals exposed to the highest concentration of chlorine for 5 or 10 minutes showed hyperplasia in the lining epithelium of the conductive airways at day 14.

The estimated LC-01 values seemed to correspond with the onset of irreversible lung damage.

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Introduction

Chlorine is produced world-wide in vast amounts and is used as a bulk material in the chemical industry. Its irritating and toxic effects on the respiratory system have been recognized for many years and, during World War I, chlorine was used as a war gas. Surprisingly, limited acute inhalation toxicity data are available concerning exposure to this compound. For rats only two studies are cited in the literature [1,2] and for mice five studies [3–7]. Using the results of these studies and additional miscellaneous data from other species (including man), Withers and Lees [8] discussed the assessment of major hazards of chlorine exposure. The recognition of the need for a better risk assessment for accidental exposure to chlorine was the reason that C.E.F.I.C. (Conseil Européen des Federations de l'Industrie Chimique) sponsored the present investigation into the acute inhalation toxicity of chlorine in rats and mice. The investigation was designed to allow: 1. The determination of LC-50 values in relation to the duration of exposure. 2. Determination of the main causes of death. 3. Estimation of LC-01 values. In addition, measurement of the respiratory responses during exposure should also allow the assessment of the acute irritation of the respiratory system at non-lethal levels and the influence of changes in the respiration on the relation between mortality, concentration of exposure, and time of exposure.

Material and methods

Chlorine was supplied on behalf of C.E.F.I.C. by AKZO, Hengelo, The Netherlands. The standard purity of the product is 99.9% Cl_2. Traces of $Fe_2(SO_4)_3 \cdot 9H_2O$ are always present.

The concentration of chlorine in the test atmosphere at the inlet of the exposure cylinders was determined colorimetrically with a chlorine monitor which was supplied and constructed by AKZO. The instrument was developed for measurement of low chlorine concentrations in the environment. To allow measurement of the high concentrations used in this study a constant flow of 0.5 l/min test atmosphere was mixed with an air flow of 100 l/min. Calibration of the chlorine monitor was obtained iodometrically.

Husbandry

The rats used in this study were SPF-treated Wistar derived rats (BOR:WISW) and were obtained from Winkelmann, Versuchstierzucht GmbH & Co. KG, Borchen, FRG. Swiss mice (Charles River CD-1 Strain) were obtained from a colony maintained under SPF-conditions at Charles River France SA, Saint Aubin les Elboeuf, France. Animals were acclimatized for at least 5 days and allocated thereafter. At the start of the study the mean weight

of the male rats was 163.8 g, that of the female rats was 130.0 g, that of male mice was 31.2 g, and that of female mice was 25.3 g.

During the observation period following exposure to chlorine the animals were housed in standard holding cages in the animal room. This room was ventilated with ca. 13 air changes per hour. Temperature and relative humidity were maintained at $21 \pm 2°C$ and 35–70%, respectively. Light was artificial with a 12 h light, 12 h dark cycle. After exposure the animals were provided ad libitum with the Institute's stock diet for rats and mice and unfluoridated tap water. During exposure animals had no access to food or water.

Experimental design

The relation between LC-50 values, concentration of exposure, and time of exposure was obtained by exposing groups of 5 male and 5 female rats to different concentrations of chlorine for 5, 10, 30 or 60 minutes. The animals were exposed to chlorine in air mixtures in a horizontally placed glass cylinder (length $\times$ diameter $= 0.90 \times 0.15$ m) with sampling ports at the inlet and at the outlet part of the cylinder. The total airflow through the cylinder was 40 l/min. This high flow resulted in a rise in concentration to 95% of the target within 20 seconds. The cylinder was fitted with an interior of perforated stainless steel plates for individual housing of ten animals.

Satellite groups of 3 males and 3 females were exposed and were killed two days after termination of the exposure for interim histopathological examination. The animals of the satellite groups were exposed in cylinders placed in parallel with the main groups.

Groups of 5 male and 5 female mice were exposed to various concentrations of chlorine to determine the LC-50 values at 10 and 30 min exposures. If appropriate, the mice were exposed in the same cylinder as the rats of the satellite groups and placed in adapted units which allowed individual housing. After exposure the animals were maintained for a 14 day observation period during which their general behaviour and health condition was checked twice a day during working days and once a day during the weekends. Body weights were measured just before exposure (day 0) and on days 1, 2, 4, 7, and 14.

After the observation period all remaining animals were killed by exsanguination under ether anaesthesia and were subjected to gross examination. The kidneys, liver, and lungs were weighed from animals killed at the end of the fourteen-day observation period (rats and mice). The nasal passages, larynx, trachea, and lungs from animals from selected exposed groups were fixed in a neutral, aqueous, phosphate-buffered 4% solution of formaldehyde for microscopic examination. Lungs were fixed by tracheal perfusion. Sections were stained with haematoxylin and eosin. In these animals differences in the effects of chlorine exposure under different exposure conditions were studied.

Respiratory measurements

One male and one female from the satellite groups which were exposed for 60 minutes received nose-only exposure to enable the measurement of ventilation. These rats were placed in modified Battelle restraining tubes, installed in a constant volume plethysmograph with a volume of approximately 2 liters and exposed to the same test atmosphere as the other rats. This volume of the plethysmograph results in an additional respiratory load of maximally 1 cm H_2O. Smaller plethysmograph volumes yield unacceptable high respiratory loads. Thoracic volume displacements were linearly transformed into pressure fluctuations. The pressure signal was transformed into an electrical signal by a Validyne pressure transducer (DP15–20) and carrier amplifier and recorded on analogue magnetic tape. After an adaptation period, recording was started 5 minutes before start of the chlorine exposure and continued until half an hour thereafter. Off-line, episodes of the recorded data were replayed on an x–t paper recorder at regular time intervals. Breath-volume and breathing frequency in these episodes were then measured manually. The minute volume per episode and the mean minute volume during the exposure were calculated from these data. Breathing pattern was also established from these paper recordings.

Time–concentration–mortality relationships

The mortality data were analysed according to the maximum likelihood probit analysis of Finney [9]. The computer program employed was designed by Dr W.F. ten Berge (DSM, Geleen, The Netherlands). The program allows introduction of three independent variables (concentration C, time T, and sex). The variables introduced can be used unaltered, reciprocal, or with logarithmic transformation. Additionally, cross-terms of the independent variables can be used in the evaluation of the response curve. Using Fieller's theorem [10] the program allows estimation of LC or LT values and their 95% confidence limits.

Results

The concentrations of chlorine together with the mortality data are listed in Table 1.

Calibration of the chlorine monitor against iodometric titration showed a linear relationship. Reproducibility of the conversion factor showed a standard deviation of 1.9% over the range from 0.5–6.0 g/m^3. Standard deviation of the concentration of exposure was better than 4%. Results of groups F and M were deleted due to instability of the concentration during the exposure.

Clinical observations

All rats were restless during exposure, their eyes were mostly closed and often irritated, especially during the 30 and 60 minutes exposures. Signs of

TABLE 1

Group code, concentration of chlorine in test atmosphere, exposure time, corresponding mortality rates and day of death

Group code	Concentration (mg/m^3) ± SD	(n)	Exposure time (min.)	Mortality rate (%)	Day of death* (number)
		Rats			
A	1654 ± —	(1)	5	0	
G	2201 ± 92	(2)	5	0	
I	2399 ± 82	(3)	5	0	
P	3485 ± —	(1)	5	0	
S	4798 ± —	(1)	5	0	
U	8241 ± —	(1)	5	0	
W	16801 ± 72	(6)	5	70	0(7)
B	1680 ± 0	(2)	10	0	
H	2186 ± 81	(4)	10	0	
J	2363 ± 81	(6)	10	0	
N	3485 ± 0	(2)	10	0	
T	4798 ± 0	(2)	10	10	0(1)
X	6519 ± 30	(6)	10	60	0(6)
E	1586 ± 47	(7)	30	0	
K	1665 ± 32	(7)	30	30	0(1) 1(1) 6(1)
C	1757 ± 35	(4)	30	50	0(2) 1(1) 3(2)
Z	1870 ± 21	(16)	30	60	0(2) 1(3) 2(1)
Y	935 ± 32	(31)	60	0	
Q	1325 ± 35	(16)	60	40	1(2) 2(1) 11(1)
O	1473 ± 19	(11)	60	60	0(3) 1(1) 2(1) 4(1)
L	1651 ± 29	(14)	60	80	0(6) 1(1) 4(1)
D	1725 ± 57	(6)	60	100	0(6) 1(3) 5(1)
		Mice			
B	1680 ± 0	(2)	10	0	
H	2186 ± 81	(4)	10	0	
J	2363 ± 81	(6)	10	30	6(1) 13(2)
N	3485 ± 0	(2)	10	40	0(2) 10(1) 13(1)
V	3826 ± 38	(3)	10	100	0(8) 1(2)
T	4798 ± 0	(2)	10	100	0(10)
R	1328 ± 55	(8)	30	40	0(1) 8(2) 13(1)
E	1586 ± 47	(7)	30	70	0(4) 8(1) 9(1) 11(1)
K	1665 ± 32	(7)	30	60	0(2) 1(2) 9(1) 11(1)
C	1757 ± 35	(4)	30	90	0(5) 10(3) 13(1)
Z	1870 ± 21	(16)	30	70	0(3) 1(2) 8(2)

Groups F and M have been removed from file due to incorrect exposure.
*0 = day of exposure. In group G the two actual values were 2136 and 2266 mg/m^3.

dyspnea were seen, both during and following exposure. Animals showed wet nares, bubble formation and nasal discharge. This was seen mainly in animals exposed to the highest concentrations. Mortalities occurred during the exposure period, as well as during the observation period. In rats most mortalities occurred within the first week with only one animal out of 56 dying in the second week of the observation period. In contrast, in mice 17 out of 59 deaths occurred in the second week of the observation period. In acute inhalation studies such delayed deaths are often the result of secondary infection. Both male and female rats and mice lost body weight during the first 2 days after exposure, which is a common finding in this type of experiment. After day 4, most animals gained body weight again but weight gain was generally low, especially in mice.

Gross pathology

Relative lung weights were generally increased with the increases showing a positive correlation with concentration and duration of exposure. Relative lung weights as a function of the estimated probit of the test groups are shown in Fig. 1. For kidneys and liver no dose-related effects were seen. At necropsy, rats sometimes showed red and dirty noses and discoloured, swollen lungs.

Histopathology

Light microscopic examination was performed on tissues from animals of five test groups listed as follows:

Group D, 100% mortality. Three rats exposed for 60 minutes to 1.7 g/m^3 which died the day of exposure were examined. Two male rats showed no abnormalities in the organs examined. The other animal, a female, showed epithelial dysplasia and cyst-like lesions with hyperplastic epithelium in the larynx and trachea and focal pneumonitis, focal hypercellularity of septa and oedema in the lungs. Since most of these changes need time to develop, it is most likely that these changes resulted from a previous infection and were not related to the exposure to chlorine.

Group K, 30% mortality. Two rats of the satellite group exposed for 30 minutes to 1.7 g/m^3 were killed at day 2. Both rats showed slight hyperplasia of the lining epithelium of the larynx and trachea and in one animal this was accompanied by squamous metaplasia. In the lung, focal aggregates of mononuclear inflammatory cells, increased septal cellularity, and squamous metaplasia of bronchiolar epithelium were observed.

Seven animals of the same group (K), killed on day 14, did not show the aforementioned changes in larynx and trachea. In the lungs of all these animals except one we observed focal aggregates of polymorpho- or mononuclear inflammatory cells. Focal oedema was observed in two animals and increased

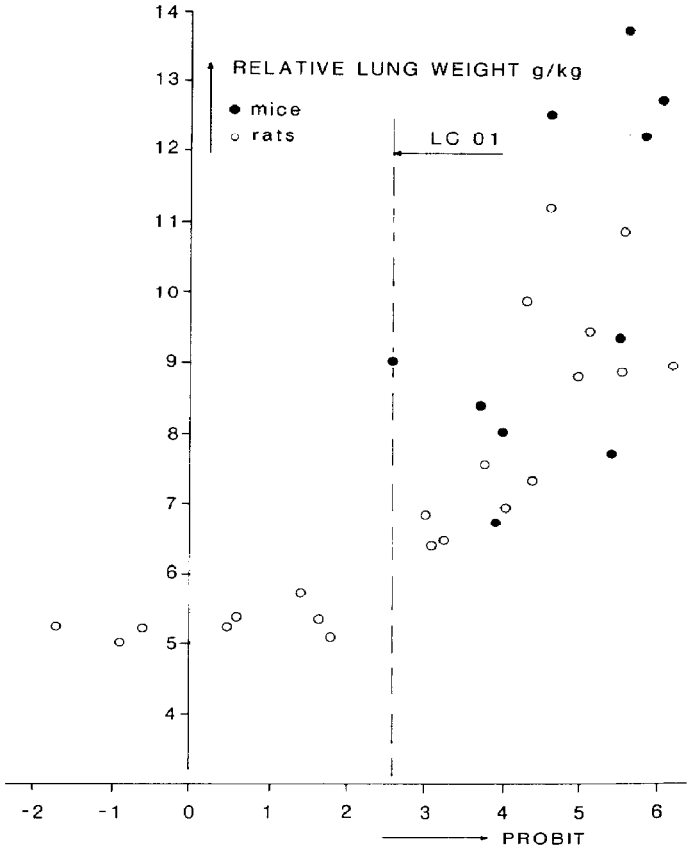

Fig. 1. Changes in relative lung weight as function of the group mortality expressed as probit value.

septal cellularity in a further two. In one of the latter pair the septal cellularity was accompanied by oedema and in the other by disorganized bronchiolar epithelium.

Group L, 80% mortality. Animals exposed for 60 minutes to 1.7 g/m^3 showed similar abnormalities as group K.

Groups W, 70% mortality and X, 60% mortality. Animals from group W, exposed for five minutes to 16.8 g/m^3, and group X exposed for 10 minutes to 6.5 g/m^3, showed basically the same histopathologic changes in the lungs as was seen in groups K and L, but effects were also seen in the nose, larynx, and trachea, indicating increased irritation responses in the conductive airways at these high chlorine concentrations.

Respiration

Respiratory responses were measured in 5 male and 5 female rats exposed to chlorine for 60 minutes. All animals responded acutely with rapid shallow breathing which lasted less than a minute. Thereafter, all animals showed apneustic breathing characterised by a low breathing frequency, a maximal inspiration and a long post-inspiratory pause. Expiration was rapid and directly followed by inspiration. In several rats the respiration pattern changed to gasping near the end of the exposure period. At approximately the same low breathing rate a maximal inspiration was directly followed by expiration and a post expiratory pause. The minute volume of these rats decreased to a value between 14 and 39% of the pre-exposure value. Animals responding at the low and high side of this distribution died. Thus two rats, where respiration decreased to 15% and 32% of the pre-exposure value, died during exposure and two rats with a decrease to 24% and 39% of the pre-exposure value in the minute volume died during the observation period.

Time–concentration–mortality relationships

Mortality data from Table 1 were used to calculate time–concentration–mortality relationships and LC-50 and LC-01 values. The relation giving the best fit to the data of the rat experiment (lowest χ^2) was:

$$P = -16.67 + 1.33 \ln C - 4.31 \ln T + 1.01 \ln C \times \ln T, \quad (\chi^2 = 15.56)$$

with P = probit response, C = exposure concentration (mg/m^3), and T = exposure time (minutes). Values of LC-01, LC-50, and the 95% confidence limits estimated with this probit relation are given in Table 2.

TABLE 2

LC-50 values of chlorine in mice and rats and LC-01 values of chlorine in rats

Time (min.)	LC-01 (mg/m^3)	95% confidence interval	LC-50 (mg/m^3)	95% confidence interval
		Rats		
5	7260	5256–9092	15949	12859–21184
10	2986	2317–3497	5642	4998–6537
30	1248	1007–1400	2033	1901–2223
60	834	644–1000	1321	1202–1423
		Mice		
10			3064	2560–3657
30			1462	1198–1671

Conversion into ppm is obtained by multiplying concentration in mg/m^3 by 0.317.

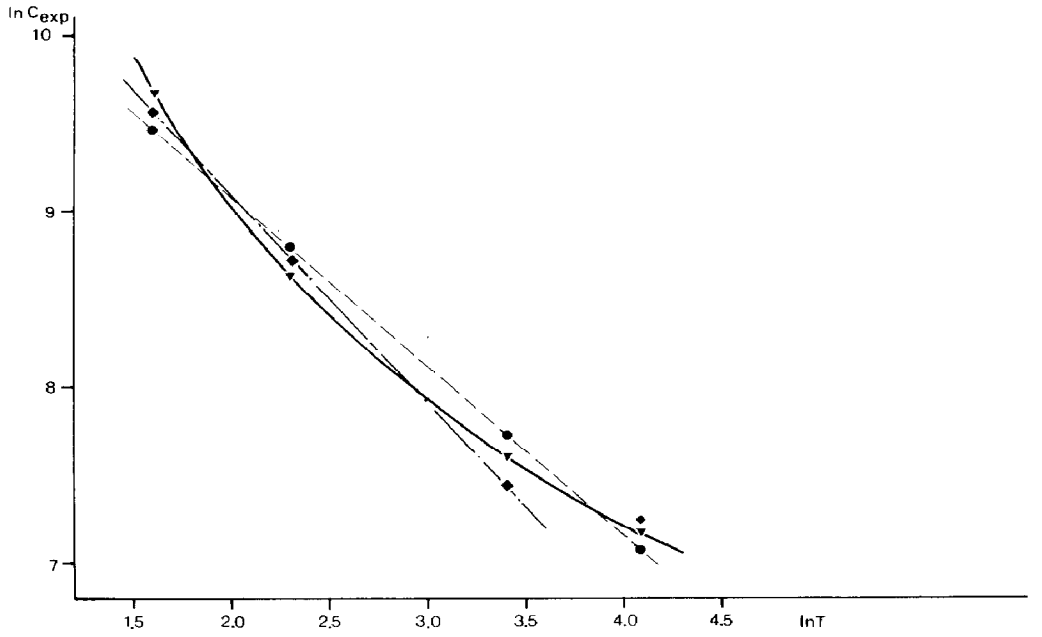

Fig. 2. Estimated combinations of ln C and ln T yielding 50% mortality or probit $(P)=5$ using different equations for data fitting. ♦ estimated LC-50 for one exposure period, • $P=5=-26.84+2.89 \ln C+2.78 \ln T$, and ▼ $P=5=-16.67+1.33 \ln C-4.31 \ln T+1.01 \ln C \times \ln T$.

The standard relation without the cross term yielded:

$$P=-26.84+2.89 \ln C+2.78 \ln T, \quad (\chi^2=24.10)$$

The difference in fit of both functions is illustrated in Fig. 2.

The LC-50 value of chlorine in mice was calculated using:

$$P=-33.74+4.05 \ln C+2.72 \ln T, \quad (\chi^2=12.97)$$

LC-50 values and their 95% confidence limits estimated with latter equation for mice are summarized in Table 2.

Discussion

Clinical and morphological observations together with lung function tests confirmed that exposure to chlorine affects the lung function and the histological integrity of the respiratory system. The clinical observation of nasal discharge with bubble formation indicates an increase in discharge of mucus from the respiratory tract. Additionally, foamy discharges usually indicate lung oedema but confirmation of this is difficult on pathological examination. Swollen lungs, also indicative of oedema, were observed in animals examined soon after spontaneous death or after the scheduled kill in the satellite groups. However,

in order to establish the presence of oedema with any certainty, the lungs have to be incised to show the superfluous fluid. Fixation of the lungs for microscopic examination involves flushing of the lungs with preservation fluid, in which the superfluous oedemic fluid dissolves, making it very difficult to detect oedema microscopically.

Mean lung weights of both rats and mice killed at the end of the observation period showed a close correlation with the probit value assigned to the test groups above the estimated LC-01 value as shown in Fig. 1. Furthermore, in these groups above the estimated LC-01 value there was a good correlation between individual relative lung weight and histopathological changes observed in the lungs. Microscopic examination of the animals in the satellite groups exposed to high concentrations of chlorine showed hyperplasia of the lining epithelium in the larynx and trachea which resolved during the fourteen days observation period. The present clinical and histopathological findings are in agreement with those described in the review by Withers and Lees [8].

Respiration

Ventilatory measurements give additional information on acute functional changes in the lung. Shortly after the start of chlorine exposure the breathing pattern changes from the normal eupnea (regular inspiration directly followed by a regular expiration) to rapid shallow breathing. This pattern is typically associated with irritation of the conductive airways (irritation reflex) which lasted in this investigation for less than a minute. After this, the breathing pattern changed to apneustic breathing (maximal inhalation directly after expiration and a long post-inspiratory pause). This breathing pattern is generally believed to be controlled by the apneustic centre of the central nervous system located in the middle and caudal pons. If this theory is correct it might have been the level of chlorine or derived pH changes in the blood or brain tissue which caused the apneustic breathing. The hypo-ventilation due to irritation of the conducting airways may also cause a decrease in blood pH, which could be responsible for the apneustic breathing pattern. The change of the breathing pattern towards a gasping type (maximal inspiration directly followed by expiration and a long post-expiratory pause) has been described as a reflex of the alveolar J receptors [11,12]. It is interesting to note that in these quoted studies the pulmonary oedema was obtained by exposing the experimental animal (cat) to a high chlorine concentration.

The decrease in minute volume to about 20% of the pre-exposure level seems to be an important and well-tuned defence mechanism. It strongly reduces the intake of chlorine while maintaining the animal at a metabolically competent level. A further decrease in ventilation however, it not possible without severe metabolic repercussions. From the ten animals in which the ventilation was measured, three animals died that were located on the high side of the ventilation distribution (minute volume 39, 32, and 24% of the pre-exposure level,

respectively). They received a relative high dose of chlorine. One animal died with a minute ventilation of only 15% of the pre-exposure level. This animal may have died primarily from hypo-ventilation. The lowest estimated LC-01 value was very close to the minimum concentration at which respiratory response was measured. At this concentration the response in the ventilation was not different from that at higher concentrations. This indicates that at the LC-01 level the chlorine is still severely irritating and is in agreement with other studies on the sensory irritancy of chlorine [7].

Time–concentration–mortality relationships

The time–concentration–mortality relationship $R=f(C^nT)$ is often transformed into the following probit function:

$$P=b_0+b_1 \ln C+b_2 \ln T \tag{1}$$

where: R = response, P = probit, C = concentration of exposure, T = time of exposure, b_0, b_1, and b_2 constants, $n=b_1/b_2$. For $b_1=b_2$, $n=1$ and eqn. (1) then describes the dose–response rule of Haber, obtained from his experiments with phosgene.

Plotting the ln C as function of ln T of points with equal probit or response should form a straight line with slope $-b_2/b_1$. However, comparison of the fitted LC-50 line with the LC-50 values as estimated individually for the different exposure times showed a systematic error suggesting a concave curvature in the ln C–ln T plot, as shown in Fig. 2. In order to obtain a better fit several variations of the probit–concentration–time relationship were applied, such as time shifts correcting the real time of exposure with a fixed period of a few minutes and using $1/T$ instead of ln T. The best results were obtained by adding a cross-term $b_3 \ln C \times \ln T$ to eqn. (1), which resulted in a considerable decrease of the χ^2 term. What does this additional cross term mean?

Rewriting eqn. (1) with the cross term yields:

$$P=b_0+(b_1+b_3 \ln T) \ln C+b_2 \ln T. \tag{2}$$

Equation (2) shows that introduction of the cross-term ln $C \times$ ln T corresponds to the introduction of a time-dependent slope in the probit–concentration relation. In Fig. 2 the 50% mortality line ($P=5$) is plotted as ln C vs. ln T calculated with the equations $P=-26.84+2.89 \ln C+2.78 \ln T$ or $P=-16.67+1.33 \ln C-4.31 \ln T+1.01 \ln C \times \ln T$. Estimates of the LC-50 are added to the figure if only rates of one exposure time are used. Note that latter points for exposure times of 5, 10, and 30 minutes are located on a virtually straight line. The response diverges from the straight line (golf club) after 30 minutes of exposure, indicating a time-dependent slope. The consideration of a time-dependent slope is important since it was the intention of this investigation to estimate the extrapolated LC-01 values at different exposure times. LC-01 and LC-50 values of chlorine in rats were calculated using

the cross-term ln $C \times$ ln T. The effect for the estimated LC-01 value using the cross-term or using the generally used probit function (eqn. 1) is shown in Fig. 3. It can be seen in Fig. 3 that the LC-01 values estimated with the cross term are higher for all times of exposure. For the 60 minute LC-01 the difference is so large that the value obtained with the conventional description falls outside the 95% confidence limits. It should be realized that the type of response function indicated in Fig. 2 (golf club) cannot be described completely by the functions available in the computer program used. As a result of this, the errors for the different time intervals are not randomly distributed using the cross-term

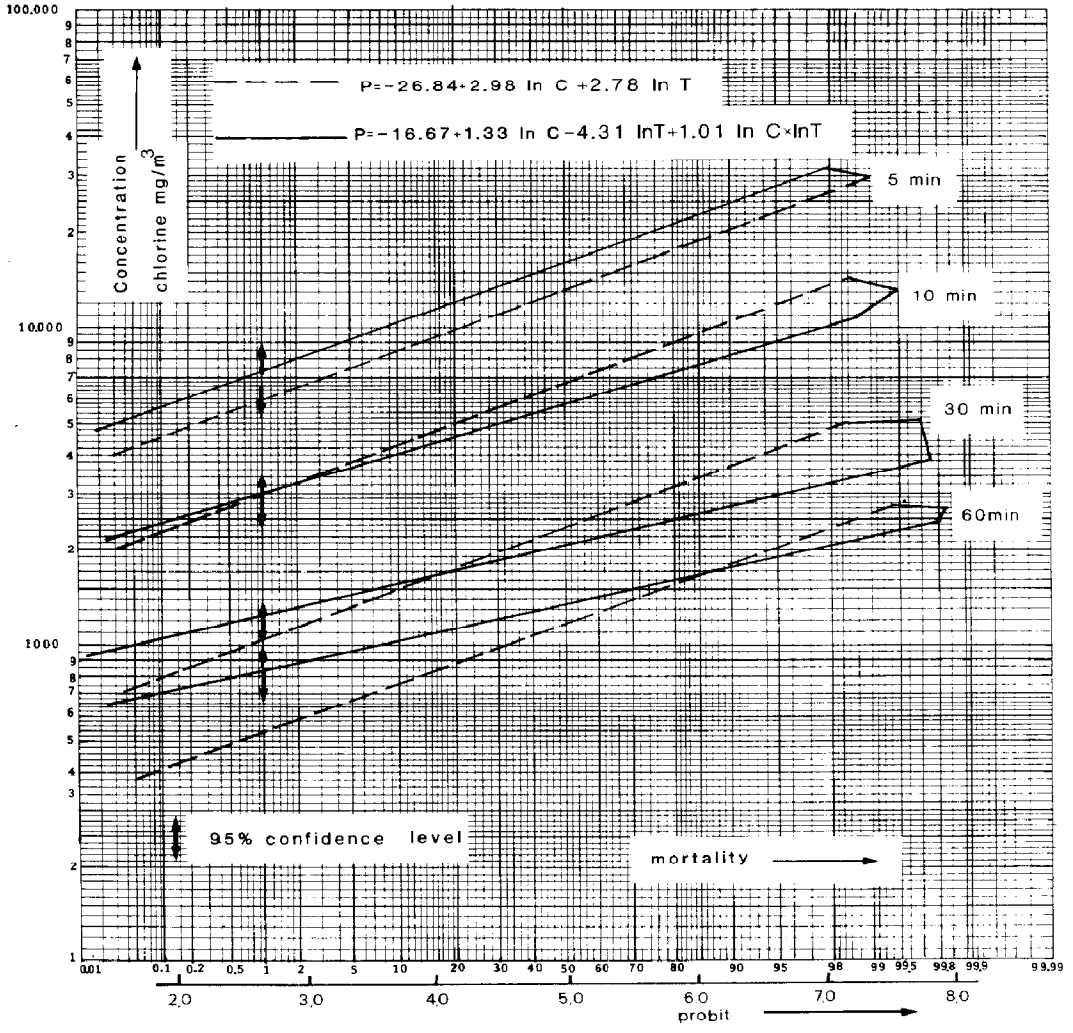

Fig. 3. Mortality or probit value as function of the concentration of chlorine for different times of exposure and different descriptive equations.

ln $C \times$ ln T, despite the considerable decrease in χ^2. Time dependency of n has been observed previously (unpublished data on H_2S) and is in agreement with uptake and distribution dynamics [13]. Uptake and distribution dynamics yield an increase in n with time of exposure if the toxic action of the compound is directly related to the concentration of the delivered compound in the target tissue. This is also true when the toxic effect is exerted by a compound which is one of the intermediate products of a non-saturated reaction pathway. In the case of chlorine this might be hydrogen chloride or hypochlorous acid. An important consequence of an increase in n with increasing exposure time is the narrowing of the concentration range between no effect and mortality after extended exposure.

No sex dependency was observed in the case of chlorine exposure.

For mice the limited amount of data did not allow investigation of time dependency in n.

Comparison with the literature

In comparison with data described in the literature, we find generally higher values for the LC-50 in both rats and mice. For rats few data are available but our 60-min LC-50 value is 0.5 to 1.5 times the values reported [1,2]. For mice more data are available. Weedon [1] reported a 30 minute LC-50 value of twice our value. Other workers reported lower values from 2 to 4 times [3–7]. The value of n reported for mice by Ten Berge et al. [14], based on Ref. 6, is slightly higher than our value. This can be explained by the longer exposure time (55 min.) in the study of Bitron and Aharonson [6]. The reasons for the lower LC-50 values reported in the literature are difficult to assess but may result from species differences. Bell and Elmes [15] investigated the influence of health status in animals repeatedly exposed to chlorine for 3 h/day. With an accumulated exposure time of 29.5 h they found an LC-50 of 369 mg/m^3 (117 ppm). For the same time of exposure we calculate an LC-50 of 433 mg/m^3 or 93.5 mg/m^3 when using the time–concentration relationships with or without a cross-term, respectively. The first value compares extremely well with the experimental data after repeated exposure. This suggests that repeated exposure results in accumulation of the effects of the single exposures, at least when the respiratory system is given no time for repair of damaged tissues. In normally bred rats Bell and Elmes found an LC-50 of 334 mg/m^3 after 17 h of accumulated exposure, where we calculate an LC-50 of 485 mg/m^3. Finally, a possible technical reason for the higher concentrations which we observed may be caused by fluctuations in the concentration during exposure. If considerable fluctuations in concentration occur, the highest concentration will often be the cause of death, but the reported mean concentration of exposure may be considerably lower. In our experiments the concentration of chlorine was continuously monitored and was found to be extremely stable, only two groups (F and M) had to be removed from the data list because of instability of the ex-

posure. The stability of the chlorine concentration might have contributed to the higher LC-50 values found in the present study compared with others reported in the literature. We conclude that: (1) The histopathological findings were in agreement with the literature, although oedema was not confirmed with certainty. (2) The LC-50 values were generally higher than described in the literature. (3) The decrease in ventilation during exposure seems to be an effective defence mechanism to decrease chlorine uptake. (4) The estimated LC-01 values seem to correspond with the beginning of irreversible lung damage accompanied by increased relative lung weight.

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