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A Study on the Combined Action of CO and HCN in Terms of Concentration-Time Products

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Summary. Acute toxicity at single and combined exposures of CO and HCN was studied on rats in terms of concentration-time product (ppm · min) necessary to kill animals (lethal CT). The animal was exposed individually to test gas in an animal chamber made of transparent plastics, and test gas was made in a gas chamber connected to the animal chamber by a wide and short piece of plastic tube. HCN was produced by addition of NaCN solution to H_2SO_4 and in case of CO exposure, various amounts of pure CO were introduced. During exposure, gas samples were frequently taken. After exposure, blood sample was withdrawn from the right side of the heart. CO concentrations in the gas and blood were determined gas chromatographically. HCN in the gas sample was measured spectrophotometrically, after being absorbed into NaOH solution in a glass vessel devised by our laboratory.

At single exposures, mean lethal CT for CO was $78{,}000 \pm 22{,}000$ and for HCN was $4{,}700 \pm 940$. In combined exposure, various combinations of CO and HCN were used. A fractional CT, defined as a ratio of CT to lethal CT, multiplied by 100, was calculated for each gas. A linear relationship between fractional CTs of HCN and CO was considered to show a simple additive action between the two gases. The sum of both fractional CTs averaged 100 ± 26. On the other hand, linear relation was not observed between blood levels of the two toxicants at death.

Key words: CO – COHb – Blood cyanide concentration – Combined action of CO and HCN

Zusammenfassung. Die akute toxische Wirkung von CO und HCN in alleiniger und kombinierter Exposition wurde an Ratten nach der Größe eines Konzentration-Zeit-Produktes (letales CT), welches erforderlich ist, um ein Versuchstier zu töten, geprüft. Einzelne Tiere wurden in einer Versuchskammer aus transparentem Kunststoff einem Prüfungsgas bis zum Atemstillstand ausgesetzt. Jedes Prüfungsgas wurde in einer durch ein kurzes und breites Plastikrohr mit der Versuchskammer verbundenen Gaskammer hergestellt. HCN wurde aus NaCN und H_2SO_4 hergestellt. Bei CO-Versuchen wurden verschiedene

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Mengen von CO in die Kammer eingeführt. Während des Aufenthaltes eines Tieres in der Versuchskammer wurden wiederholt Gasproben entnommen. Am Ende eines Versuches wurde eine Blutprobe aus der rechten Herzkammer entnommen. CO-Konzentrationen in Gasproben und COHb wurden gaschromatographisch bestimmt. HCN in Gasproben wurde spektrophotometrisch bestimmt, nachdem Zyanid in NaOH-Lösung in einem von unserem Laboratorium entwickelten einfachen Glasgefäß absorbiert wurde. Das durchschnittliche letale CT (ppm · min) bei alleiniger Exposition war 78000 ± 22000 für CO und 4700 ± 940 für HCN. In kombinierten Versuchen von CO und HCN wurde ein relatives CT, welches als ein Verhältnis von einem CT zum betreffenden letalen CT definiert wurde, für beide Gase berechnet. Aus einer linearen Beziehung zwischen relativem CT beider Gase wurde angenommen, daß es eine einfache additive Wirkung zwischen CO und HCN gibt. Zwischen Zyanid- und COHb-Konzentrationen im Blut wurde keine lineare Beziehung nachgewiesen.

Schlüsselwörter: CO – COHb – Zyanid – Blutzyanidkonzentration – kombinierte Wirkung von CO und HCN

Introduction

Fire deaths are considered to be mainly due to inhalation of toxic combustion products. Introduction of a variety of new synthetic materials into daily use has made the composition of combustion products far more complicated in comparison with that of fires in earlier days. The potential hazard of these materials at fires has been studied by many investigators. Of combustion products carbon monoxide (CO) and hydrogen cyanide (HCN) are practically the most important, because of their high toxicity and occurrence in almost all fires, CO being produced by incomplete combustion of carbon-containing materials and HCN from materials having nitrogen. In real fires, simultaneous production of many toxic gases is not uncommon, and among them co-existence of CO and HCN is considered to be very serious, and toxicologically also very important [1, 2]. However, there has been only a limited number of experimental reports on the combined action of these toxicants except for those by Smith et al. [3, 4] and by Yamamoto [5]. The reasons for scarcity of available information on this subject probably seem to lie in the fact that accurate preparation of HCN with a desired concentration and rapid determination of it are not as easy as those of CO, which is called an indicator component in combustion toxicology experiments [6]. Smith et al. [3, 4] exposed rats to single gas and gas-mixture and recorded time to intoxication (physical incapacitation and/or death) and reported that there is a simple additive action between the two gases. In a further study based on the comparison of the doses necessary to produce intoxication, they reached the same conclusion (pp. 137–138 of preprint from aerospace medical association scientific program, San Francisco, Calif., USA, on April 28 · May 1, 1975). Yamamoto [5], on the other hand, having studied the combined action on the basis of blood level analyses of the respective toxicants, could not find evidence for additive action.

Recently, we have developed a simple method of collecting a gas sample for HCN determination and applied the method to the present study.

Materials and Methods

HCN Concentration Determination in a Gas Sample

As a sampling vessel, a 30 ml capacity of Erlenmyer flask with two straight side tubes, each of which can be closed by turning a glass stopcock, was used. The orifices of these side tubes into the flask chamber are opposite. Prior to starting a sampling procedure, 2 ml of 0.1 N NaOH solution was put into the flask and the vessel was closed with a silicon rubber stopper. After inside pressure of the vessel had been reduced to a subatmospheric level by aspirating with a large-capacity glass syringe connected to a side tube, a gas sampler containing accurately 15 ml of sample was attached to a side tube and the sample was completely transferred into the flask by opening a stopcock. Immediately after completion of sample transfer, the stopcock was opened for very short time for equating inside pressure with ambient pressure, and the flask was manually shaken for short period. After 2 h standing at room temperature, during which cyanide dissolves into the solution, the solution was determined for cyanide by the method of Feldstein et al. [7] and the original HCN concentration in the gas was calculated.

Exposure Method

Male Wistar rats, each weighing about 200 g, were used.

The apparatus for exposure consists of two, transparent plastic boxes of the same shape and capacity (measuring 15 cm × 15 cm × 11 cm) connected with each other by a piece of flexible plastic tube (9 cm long and 1.2 cm wide). Each box has a removable lid and has holes in every side walls and upper wall.

After an animal had been put into a box I (termed as animal chamber), test atmosphere was prepared in situ. With CO exposure, 60–100 ml of pure CO was manually introduced into box II (termed as gas chamber) by a syringe through a side hole. HCN was produced in the gas chamber by addition of NaCN to H_2SO_4. From a buret inserted into the gas chamber through an upper hole, about 10 ml of NaCN solution was added into a dish containing 1 ml of concentrated H_2SO_4. The delivery tip of the buret was positioned directory over the dish placed on the floor. Varying concentrations of NaCN solution were used. It took about 30 s on average for an addition. In combined exposure with CO and HCN, introduction of CO and addition of NaCN solution were started almost simultaneously. During the first few minutes, the atmosphere in the chamber was stirred vigorously by pushing in and out the plunger of a syringe connected to the sampling hole of box I. Gas samples were withdrawn by glass syringes separately for CO and HCN. Frequency of sampling was maximally eight times for each gas, intervals of sampling being shorter at early stage of exposure. A flexible plastic bag was connected to the side hole opposite to the CO-introducing hole to keep the inside pressure from being reduced to a subatmospheric level by repeated sampling procedures. One sample volume was about 20 ml for CO and accurately 15 ml for HCN. The CO concentration was determined by injecting the sample into the gas chromatograph via the heated gas sampler and by comparing the peak height of the sample with that of the reference gas similarly treated [8].

In all of the single-HCN and combined exposures and in many of the single CO-exposures, animals were made to inhale the gases until respiration had stopped ultimately. After thoracotomy, blood sample was taken from the right side of the heart by a heparinized syringe. The COHb concentration was determined gas chromatographically [8]. Blood and a magnetic bar are placed into the main chamber and potassium ferricyanide solution is placed into the side chamber of a glass vessel. A helium-containing saran bag is connected to one of the side tubes. After sealing the vessel, the degassing solution is transferred into the main chamber by tilting the vessel, and the mixture is stirred by a magnetic stirrer. CO released is drawn manually with helium into a glass syringe connected to the other side tube and the sample is injected into the gas chromatograph. The procedure is repeated at about 3 min intervals until only a negligible amount of CO appears on the chromatogram. The COHb concentration was calculated as a ratio of the CO-content to the CO-binding capacity of the total Hb in the sample, multiplied by 100.

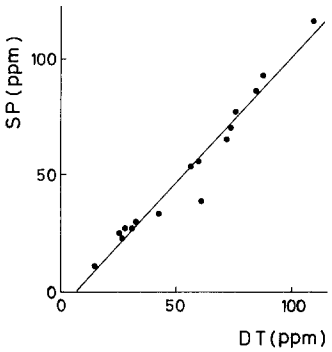

Fig. 1. A comparison of cyanide concentrations in gas samples by the present method (*SP*) and Kitagawa's detector tube method (*DP*). Linearity is present between them. The linear regression line of SP on DT is drawn

Table 1. The summarized data of the single-exposure experiments

	Survival time (min)	Peak concentration (ppm)	Lethal CT (ppm · min)	Blood concentration
HCN	16.1 ± 5.3	197–626	4,700 ± 940	2.82 ± 0.48 (μg/ml)
CO	8.5 ± 3.4	7,100–20,000	78,000 ± 22,000	84 ± 4%

A lethal CT was calculated from the concentration-time curve for each rat by integrating an area under the straight line drawn through each point from zero to death time

Results

HCN Determination Method

The coefficients of variation were small, ranging 2.2%–3.9%. The relation between sample volumes introduced (5, 10, and 15 ml) and cyanide concentrations in the solution was linear. There was not significant difference among three standing times (2, 2.5, and 3 h) in terms of cyanide concentrations. The present method was compared with Kitagawa's detector tube method, which enables HCN concentration in a test gas to be read out by naked eye by comparing the length of the stained part of a detector tube with a standard chart. The comparison tests on 15 samples ranging in concentrations 10–110 ppm, shows (Fig. 1) that the values of the two methods approximated well.

Single HCN Exposure

HCN concentration in the chamber rose steeply after addition of NaCN and thereafter increased gradually, reaching peak value. The time to reach peak value became longer as the peak value became higher. About 80% (22 of 27) of rats died within a 20-min exposure and the rest of the animals were succumbed for a further 10-min exposure. Table 1 gives the summarized data. There was a negative correlation between the peak concentration and the length of survival time, the correlation coefficients for 27 rats being -0.69 ($P < 0.01$).

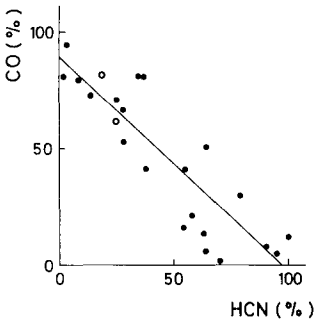

Fig. 2. A relationship between fractional CT_{CO} and fractional CT_{HCN}. A fractional CT of each gas was defined as a ratio of CT to lethal CT, multiplied by 100. A lethal CT_{CO} was calculated for individual cases by using the linear regression equation (refer to the text), because lethal CT_{CO} was linearly dependent on the length of ST. The same procedure was also tentatively applied to the two open circles in this figure, the survival time of which having lain outside the range used for calculating the regression equation (21.0 and 24.5 min vs. 18.5 min). The distribution of the points is considered linear. The linear regression line of CO and HCN is drawn

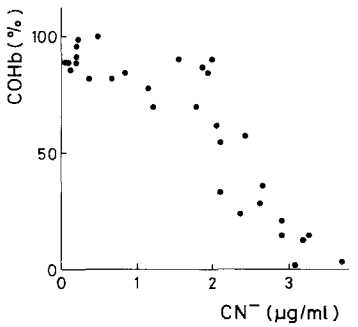

Fig. 3. A relationship between blood cyanide and COHb concentrations at the animal's death. The relation is not considered linear

Single CO Exposure

Concentration-rise profile of CO was the same as that of HCN. With CO, the time to reach peak level was much shorter. After peak levels were reached, concentrations decreased gradually. The experiment was terminated at 20 min, even if the animal was still alive, because judging from the behavior of the animal, it was not expected that the animal would have died for further exposure. The data from the rats surviving the 20-min exposure were excluded.

Table 1 gives the summarized data. The COHb values lay in relatively narrow limits. A significant negative correlation was present between the peak concentration and the length of survival time ($r = -0.67$, $N = 32$), on the other hand, there was a significant positive relation between lethal CT_{CO} and the length of survival time (ST), the linear regression line of lethal CT_{CO}(Y) on the length of ST(X) being $Y(\times 10^3\ \text{ppm} \cdot \text{min}) = 5.1X(\text{min}) + 35$. Lethal CT_{CO} did not correlate linearly with COHb concentration.

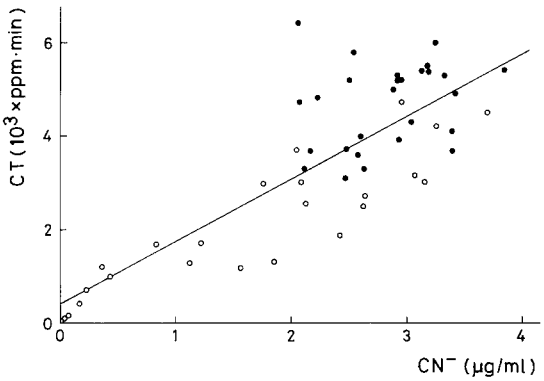

Fig. 4. A relationship between CT_{HCN} and blood cyanide concentrations (CN^-). The figure was drawn from the pooled data (open circle: combined exposure, closed circle: single exposure). The linear regression line of CT_{HCN} on CT^- is drawn

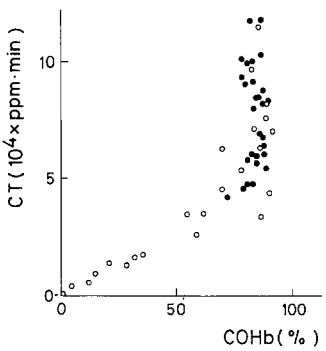

Fig. 5. A relationship between CT_{CO} and COHb concentration. The figure was drawn from the pooled data (open circle: combined exposure, closed circle: single exposure). When the level of COHb exceeds 75%, the variation of the CT becomes greater

Combined Exposure

In all, 32 rats were exposed to varying combinations of CO and HCN until they died. The time to death ranged between 5 and 24 min, and peak concentrations of CO and HCN ranged between 500 and 10,500 ppm and between 40 and 600 ppm, respectively. In 24 of 32 experiments, CT values were calculated for each gas, whereas in the remaining eight cases only blood COHb and cyanide concentrations were determined.

Figure 2 shows a relation between fractional CTs of CO and HCN, a fractional CT being defined as a ratio of CT to lethal CT, multiplied by 100. As Fig. 2 shows, the distribution pattern was considered to be linear, although variability was considerably great. The sum of both fractional CTs averaged 100 ± 26. The relation between COHb and cyanide concentrations at animal's death is graphed in Fig. 3. Linear relation between them was not observed, many points lying above a hypothetical straight line (not drawn in Fig. 3) connecting a point corresponding COHb 90% and one corresponding cyanide 3.5 μg/ml.

A relation between CT_{HCN} and blood cyanide concentration is shown in Fig. 4. The figure drawn from pooled data (single and combined exposures) shows a significant linear relation ($r = 0.80$, $N = 51$). The same plot for CO is in Fig. 5. The relation was considered linear when COHb concentration was under 75%, on the other hand, when it exceeded 75%, the range of CT became so wide that the ralation was not considered to be linear.

Oxygen concentrations were above 18% during exposure in all the experiments.

Discussion

In toxicology, it is essential to clarify quantitatively a relationship between dose and response. Setting aside the cases in which solid or liquid substance is used, it is difficult to define the dose actually absorbed by animals when a test material is administered in a gas form, particularly so when ventilation changes during exposure. So far, indices "CT" (concentration-time product) and "incapacitating or lethal dose" [4] have been used to indicate the dose in inhalation experiment. The latter term "incapacitating or lethal dose" is a kind of modified CT and means a CT multiplied by a factor depending on the body weight of the experimental animal. Since body weight of the rats in the present study lay in a narrow limit around 200 g, CT values were not corrected for body weight. There is criticism on the use of CT values [9]. It says that CT can be proportional to the toxic effects only within very narrow limits in both C or T. The criticism seems to apply to very low concentrations of CO and HCN. However, it does not hold for the present study. Because the concentration used in the present study have covered wide ranges of concentration for respective gases. Although CT necessarily overestimates the dose actually absorbed by the exposed animal, and the degree of overestimate becomes higher when respiration is impaired at a late stage of exposure, CT is still considered to give a good estimate of the dose retained by the animal.

Any intoxication symptoms appearing early during exposure can become a more practical indicator [3, 10], because such symptoms may be related with an extent to which escape ability is impaired and the degree of overestimate of CT will be much smaller with such indices than with death.

The term such as "MST" (mean survival time) [11] and LC_{50} could not be applied to the present study, because these terms can be employed only on the premise that gas concentration was being kept constant throughout exposure.

If CO and HCN act independently without interaction and only simple additive action is present between them, the sum of fractional CTs will be unity (100%) and the relationship between them will be linear. As Fig. 2 shows, the results was considered to support the above hypothesis. The present result agreed with that of Smith et al. [3].

On the other hand, when the blood data are analyzed by the method described in the previous report [5], the data in Fig. 3 have to be interpreted as showing an interaction between CO and HCN, the distribution pattern in Fig. 3 being similar to that in the previous report. The cause of the discrepancy between a conclusion based on gas-concentration analysis and one based on blood level analysis remains to be clarified, however the statement given in the previous report [5], that the

interaction of CO and HCN can be also analyzed in terms of blood levels, seems to need to be rechecked.

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