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Acute Combined Effects of HCN and CO, with the Use of the Combustion Products from PAN (Polyacrylonitrile) – Gauze Mixtures

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Akuter Kombinationseffekt von HCN und CO durch Verbrennungsprodukte von PAN–Gaze Verbindungen

Summary. The combustion product from gauze-PAN (Polyacrylonitrile) mixture was used as a material for the evaluation of the combined effects of CO and HCN. Rats and mice were exposed for 30 min. to the combustion products. In mice experiments, the time at which animals turned laterally and were killed, was measured. In experiments with rats, blood COHb and cyanide determinations were made, in addition to the observation of the behavior.
Exposure room gas concentrations (O_2, CO_2, CO, HCN) were determined and temperature in the combustion room was measured during heating. Preliminary experiments with PAN, ranging from 2g to 10g, on mice showed that HCN is responsible for the toxicity of the combustion products from this fiber. In mixture experiments all animals died during exposure. In experiments with mice (8g of total weight of mixed sample), animals in groups with larger proportion of PAN turned over and died earlier and this was explained by higher HCN concentration. In rats experiments (10g), this held true for severe impairment, however, as to the death time, this was not the case. There was a long interval between severe impairment and death in groups with larger proportion of PAN. The larger amount of HCN was produced as temperature rises.
A linear relation between COHb concentration and CT (concentration-time) product was shown. There was not a linear relation between blood COHb and cyanide levels. Judging from the blood analyses data, it seemed probable that there is neither an additive nor a synergistic action between CO and HCN with respect to lethal effects.

Zusammenfassung. Bei Verbrennungsgasen, die durch Erhitzen verschiedener Kombinationen von Gaze und PAN (Polyacrylonitrile) entwickelt wurden, wurde der Kombinationseffekt von CO und HCN geprüft. Ratten und Mäuse wurde 30 Minuten lang den Gasen ausgesetzt. Als Kriterien für die Toxizität wurde die Zeit bis zum Eintritt schwerer Vergiftungserscheinungen (die Seitenlage der Tiere) und des Todes gemessen. In der Kammer, in der die Tiere den Gasen ausgesetzt wurden, wurden die O_2-, CO_2-, CO-, und HCN-Konzentrationen gemessen, bei Ratten COHb- und die Cyanid-Konzentrationen im Blut. Vorversuche an

Mäusen mit PAN allein bestätigten, daß die Toxizität der Verbrennungsgase HCN zugeschrieben wird. In Versuchen, bei denen Tiere den Gasen aus verschiedenen Kombinationen von Gaze und PAN ausgesetzt wurden, starben alle Tiere innerhalb von 30 Minuten. Schwere Vergiftungserscheinungen und Tod der Tiere (außer Tod der Ratten) traten früher in Versuchsgruppen mit höheren Anteilen von PAN ein; dies ist höheren HCN-Konzentrationen von diesen Gruppen zuzuschreiben. Es gab lange Zeitspannen zwischen Seitenlage und Tod von Ratten in obengenannten Gruppen.

Eine lineare Relation wurde zwischen COHb Konzentration und KT-Wert (das Produkt aus CO-Konzentration und Zeit) aufgezeigt. Zwischen COHb- und Cyanid-Konzentrationen im Blut wurde keine lineare Beziehung nachgewiesen. Aus obengenannten Giftanalysen im Blut erscheint es unwahrscheinlich, daß es eine Potenzierung zwischen HCN und CO in bezug auf tödliche Folgen besteht.

Key words. HCN – combustion product from PAN, CO – combustion product from gauze, combined effects of HCN and CO.

Introduction

Fire deaths are mainly caused by inhalation of toxic combustion products. Introduction of various materials which are the products of modern chemical industry into daily use has made the compositions of the combustion products far more complicated in comparison with those in fires before. The potential hazard of these materials at fires has been investigated by many workers. Recently reactive phosphate fire retardant is remarkable for its extreme high toxicity [1]. Of the combustion products carbon monoxide (CO) and hydrogen cyanide (HCN) are practically most important owing to their very high toxicities and occurrences in almost all fires, CO being produced by incomplete combustion of carbon-containing materials and HCN from materials with nitrogen. In real fires simultaneous liberation of many toxic gases is not uncommon and among them that of CO and HCN is considered to be the most serious and toxicologically very important. There is only a limited number of investigations into combined effects of these toxic gases. The reasons for scantness of available information on this subject probably lie in the difficulties, with which HCN gas is accurately prepared and rapidly determined. On the other hand CO is sometimes called an indicator component in combustion experiments [2], since it can be accurately and continuously determined. From the previous combustion experiments with fibers it became apparent that some fibers without nitrogen (gauze and cotton) produce large amount of CO, and that from fiber such as polyacrylonitrile (PAN) considerable amount of HCN is released, the amount of CO being far less from this fiber [3]. Therefore, it was considered that an exposure of animals to the combustion products from various combinations of gauze and PAN can serve as a model experiment for the combined effects of CO and HCN.

Materials and Methods

Male Wistar-strain rats each weighing about 200 g, male dd-strain mice each of about 20 g were used. Gauze (the Japanese Pharmacopoeia) and PAN (from Nippon-Boen-Kyokai) were cut to pieces of about 1.5 cm x 1.5 cm and mixed. Experimental apparatus and methods were virtually the same as those of the previous report [3] with slight exceptions and only brief account is given in the present report as follows. Combustion was carried out in a 30x30x50 cm, transparent box of acrylic resin. The sample in a cylindrical stainless-cage was heated with an electric heater of

300w. The smoke was lead through a plastic tube to a transparent plastic box (36x36x10cm), in which animals in compartmentalized stainless-cage were exposed to the combustion products. In each run, 2 rats were exposed. In mice experiments, 9 mice were used at a time. The combustion product was exhausted from the exposure room through a plastic tube and small portion of it was carried to an infrared CO analyzer by means of an air pump at a rate of 1.5l/min. Exposure time was 30 min and the death of the animal was judged from cessation of respiration. In addition to the death time, the time at which mice turned laterally and rats were severely impaired, was measured. For a judgement of severe impairment, following criteria were used (1). The rat's inability to advance only to lay itself down flat, to maintain normal posture, to respond to external stimuli, and (2) convulsion. Detailed account has been given in the previous report (3).

The temperature during heating was continuously monitored with a thermoelectric thermometer (Yokogawa Electric Company) and exposure room O_2 and CO concentrations were determined continuously with a Beckmann O_2 analyzer and the infrared CO analyzer (Horiba), respectively. The gas sample for exposure room HCN determination was collected in a Polyvinylidenechloride (PVC) bag (2300ml) attached to the outlet of the infrared analyzer, and after subsequent bubbling of it into 1000ml of 0.1 N NaOH solution, its cyanide level was determined. HCN concentration was expressed in terms of mean concentration during sampling time by using gas flow rate and sampling time. CO_2 determination was made by volumetric Roken-type gas analyzer on small portion of the sample in the PVC bag. In experiments with rats, blood was taken from heart with heparinized syringe and COHb concentrations were determined according to the method of van Kampen et al. [4] with slight modifications and the cyanide determinations of whole blood and NaOH solutions followed procedures of Feldstein et al. [5] with slight modifications. Also in the present study, HCN inhalation experiment was conducted on the rats. The ways, by which HCN was prepared and the rats were exposed to the gas, were virtually the same as the previous study [3], but the animals were made to inhale the gas till their deaths.

Results

1. Acute Toxicity of the Combustion Products from PAN on Mice

The results of the toxicity studies on the combustion products from PAN ranging from 2 g to 10 g are given Table 1. The sample weight had decreased to 75–80% of the initial weight after heating and this agreed with the previous results. CO concentration increased with increasing sample weight, however, the highest concentration did not attain the level of 0.1% at the time of the animal's death. The lowest O_2 and the highest CO_2 concentrations were the levels of 19% and 1%, respectively. In experiments with sample weights of 8 g and 10 g, animals were killed earlier than in experiments with less sample weights, and this earlier death in PAN 8 g and 10 g groups correlated well with higher HCN concentrations attained rapidly in these experimental groups.

2. Acute Toxicity of the Combustion Products from PAN-Gauze Mixtures on Mice

The results are tabulated in Table 2 and exposure room HCN concentrations are shown in Fig. 1. As clearly seen from Table 2, the combustion products of groups with PAN more than 4 g, P8GO, P6G2, and P4G4, turned aside and killed the mice earlier than those of P2G6 and POG8 groups. The higher toxicity of the first group can be considered due to higher HCN level. There was not significant difference with respect to the highest HCN concentrations between P8GO and P2G6 groups, however HCN of P8GO group attained its highest level earlier and this probably has induced earlier deaths of animals in this group.

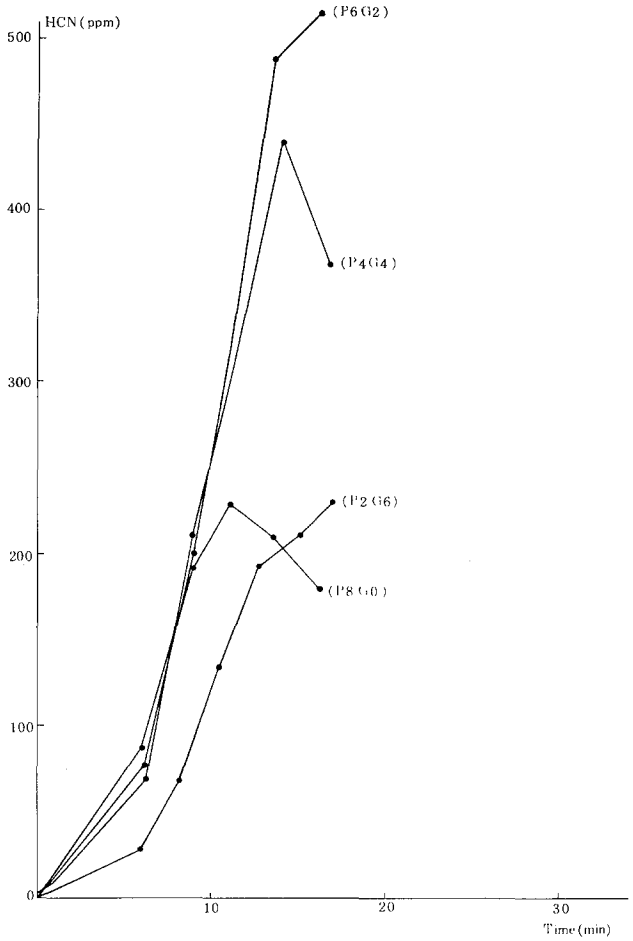

Fig. 1. Exposure room HCN concentration in experiments with PAN-gauze mixtures. The results of one experiment are given. Ordinate indicates HCN concentration in ppm, and abscissa time after heating. HCN concentration determination was made, after samples collected into the PVC bags were bubbled into 0.1 N NaOH solutions. HCN concentration is expressed in terms of mean concentration during sampling. In parenthesis experimental group is given

3. Acute Toxicity of the Combustion Products from PAN-Gauze Mixture on Rats (Table 3).

All rats died within exposure. Rats in groups P8G2, P6G4, and P4G6 were severely impaired earlier than those in other groups. As to the death time, that of P8G2 and P6G4 was the earliest. The interval between severe impairment and death was relatively short in groups P2G8 and POG10 compared with groups consisting of larger proportion of PAN. Exposure room CO concentrations were high in groups consisting of large proportion of gauze and O_2 concentrations were low in these groups.

The relation of COHb concentration with concentration x exposure time (CT product) is graphically presented in Fig. 2. The CT product was obtained by integrat-

Table 1. Acute toxicity data of the combustion products from PAN on mice. PAN ranging from 2g to 10g was heated with an electric heater. The time, at which mice turned laterally and died, was measured. In this table, data are expressed in terms of the mean time. The numerals in the 1st and 2nd parentheses represent the rates of animals turned laterally and died, respectively. The figures in the 3rd and 4th parentheses show the time, at which peak HCN concentration was attained

Exp. groups	Lateral turning (min)	Death (min)	Highest temperature (°c)	Highest HCN in the exposure room (ppm)
PAN 10g	10.8(18/18)	13.9(18/18)	490–550	288(18)–348(21)
PAN 8g	10.7(17/17)	14.4(17/17)	510–520	275(15)–337(14)
PAN 6g	13.8(18/18)	17.1(18/18)	480–500	137(18)–209(26)
PAN 4g	17.8(14/18)	26.0(6/18)	490–500	122(18)–178(25)
PAN 2g	(0/9)	(0/9)	435	37(18)

Table 2. Acute toxicity of the combustion products from various combinations of PAN and gauze mixtures on mice. PAN-gauze mixtures with varying proportion (total weight 8 g) were heated. Detailed explanation for each column is given in the legend for Table 1

Exp. groups	PAN	gauze	Lateral turning (min)	Death (min)	Highest temperature (°c)
P8GO	8	0	10.2	13.6	455–500
P6G2	6	2	10.2	13.1	573–650
P4G4	4	4	10.2	13.4	610–640
P2G6	2	6	12.4	16.1	610–630
P0G8	0	8	13.4	17.1	580–610

	Highest CO(%)	Highest HCN(ppm)	Lowest O_2 (%)
P8G0	0.08(18)	230(11)–280(13.5)	19.0–20.3
P6G2	0.25(18)–0.28(15)	384(16)–520(16)	19.1–19.7
P4G4	0.65(17)–0.70(17)	404(15)–440(14)	18.0–18.5
P2G6	0.98(18)–1.07(19)	209(18)–230(17)	16.8–18.0
P0G8	1.40(15)–1.48(17)		16.7–16.8

Highest CO_2 concentration, which was given by P0G8 group, did not exceed the level of 4%.

ing the area under the CO concentration-versus-time curve from start of the experiment till the time of the respective animal's death. Fig. 2 shows a linear relationship between two parameters (correlation coefficient 0.99).

Summarized data for the combustion experiments and for acute HCN inhalation test are graphically represented in Fig. 3. The cyanide concentrations in the present study were considerably higher than those of the severely impaired rats in the previous reports. This probably shows that considerable amounts of HCN was further inhaled by severely impaired animals. As clearly seen from Fig. 3, the relation between both parameters was not linear, and the application of the linear regression method was considered to be inappropriate.

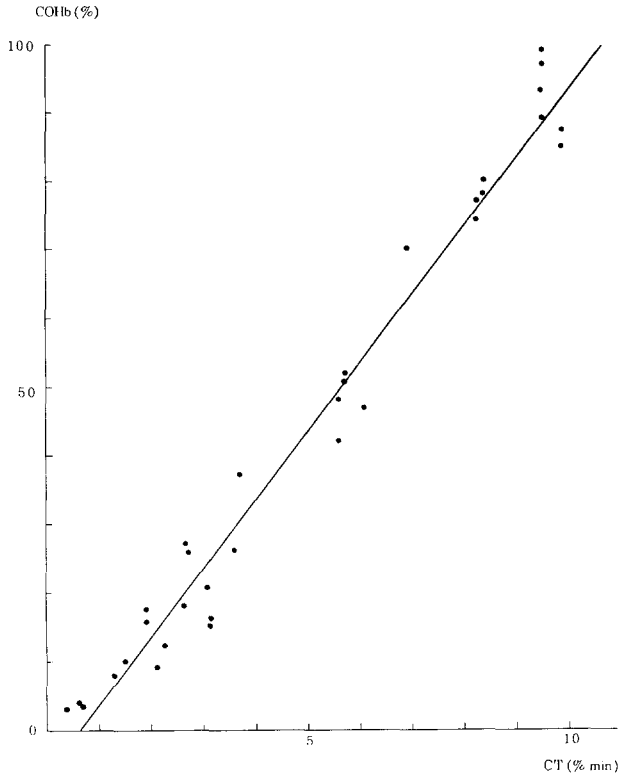

Fig. 2. A relation between COHb concentration and CT (concentration-time) product. The data were obtained from experiments with rats exposed to the combustion products from PAN-gauze mixtures. Ordinate indicates COHb concentration, and abscissa CT product. The CT product was obtained by integrating the area under the CO concentration curve from start of the experiment till the time of respective animal's death. In all, 38 pairs of comparative determinations were made ($r = 0.99$). The solid line is the fitted linear regression of COHb on CT

Discussion

It has been confirmed also in the present experiment that HCN is responsible for high toxicity of the combustion products from PAN. In mice experiments the first manifestation of severe intoxication was convulsion, which was immediately followed or simultaneously accompanied by lateral turning. With increasing amount of PAN, the occurrence of the symptom became earlier. Since there was not significant difference with respect to CO, CO_2, and O_2 concentrations among experimental groups, it was confirmed that this intoxication is due to HCN. The possibility that other gases than HCN might be responsible for high toxicity of PAN under the present conditions could be denied on the basis of the results of the acute HCN inhalation test. There was not significant difference in blood cyanide levels at the time of death between the rats exposed to the combustion products from this material and those to HCN gas. The same held true in the previous experiments, in which severe impairment was used as an index for toxicity [3]. Although there still remains uncertainty about a detailed thermal decomposition mechanism of PAN [6], it seems probable that the production

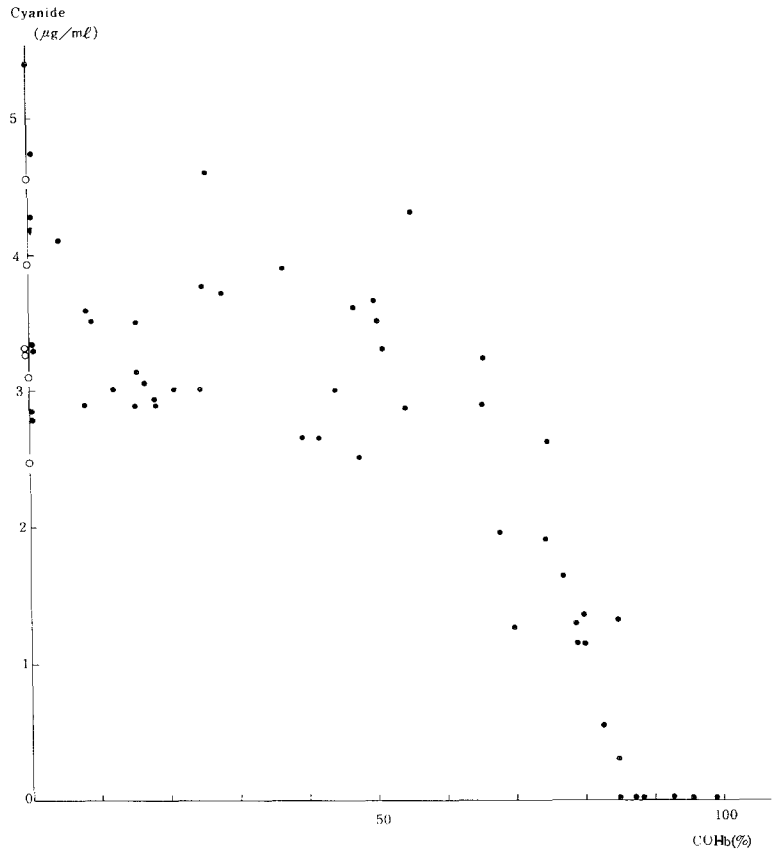

Fig. 3. The scatter diagram of the blood cyanide and COHb concentrations of rats. Ordinate indicates blood cyanide level, and on abscissal scale COHb concentration is given. In all, 54 pairs of comparative determinations were made. On the whole there is no linearity between two parameters. Open circle represents the blood cyanide level of the rats exposed to HCN gas

of HCN from PAN is temperature-dependent, increasing as temperature rises, and that at temperature attained in the present study other toxic materials are produced only poorly. The less liberation of HCN in P8GO group compared with P6G2 and P4G4 groups shown in Table 2 is probably due to the lower temperature during heating.

Since CO can be determined continuously with accuracy and ease, it is sometimes called the indicator component in the combustion experiments. As the toxicity of CO is mainly by the formation of COHb, it will be much convenient if COHb concentrations of animals under exposure can be estimated from CO concentration. Fig. 2 shows that the CT product serves as a good index for an estimation of the blood COHb level of the animals.

Combined effects of CO and HCN can be studied probably best by exposing the animals to various HCN-CO mixtures, and by comparing the LC50 values for one gas with and without another gas. However, since it is practically very difficult to prepare accurately CO-HCN gas mixtures with desired composition, as stated previously, and toxicities of both gases are not due to their metabolites, blood analyses were employed for the study of the combined actions of HCN-CO. As seen from Table 3, there were

Table 3. Acute toxicity of the combustion products from PAN-gauze mixtures on rats. Varying combinations of PAN-gauze (total weight of 10 g) were heated. The numerals in the parentheses at the column of COHb% represent the number of the animals. Detailed explanation for another column is given in the legend for Table 1

Exp..groups	PAN	Gauze	Severe impairment (min)	Death (min)	COHb (%)
P10G0	10	0	11.0	17.7	under 5%
P 8G2	8	2	8.8	14.9	14(8)
P 6G4	6	4	9.6	14.4	24(8)
P 4G6	4	6	9.0	15.5	53(10)
P 2G8	2	8	12.8	15.5	78(8)
P 0G10	0	10	13.2	15.5	92(6)

	Highest CO(ppm)	Lowest O_2(%)
P10G0	0.08–0.11	19.3–20.2
P 8G2	0.26–0.37	18.8–20.5
P 6G4	0.53–0.60	18.4–19.3
P 4G6	0.79–0.98	18.0–18.8
P 2G8	1.28–1.42	16.7–17.0
P 0G10	1.60–1.73	15.8–16.6

Highest CO_2 concentration, which was given in P0G10 group, did not exceed the level of 4%

significant differences among experimental groups with PAN in regard to O_2 concentration. The highest group was P10GO, groups P8G2, P6G4, and P4G6 came next, and P2G8 came last. At lower O_2 atmosphere CO intoxication will develop rapider than at normal O_2 atmosphere and therefore, less inhalation of HCN may result within exposure. On the ground of the above consideration, the correction for cyanide concentration may be necessary in groups with lower O_2 concentration. However, even if such correction was made, the distribution of the points in Fig. 3 would not change on the whole. Cyanide level decreased gradually with increasing COHb level, and steep lowering occurred at COHb levels above 70%.

The fact that the group of the points curved on the whole made the application of the linear regression improper. If there is an additive action between HCN and CO, a linear relation should be expected between blood levels of both parameters. This was not the case with the present study. The possibility of synergism could be also excluded from the shape of the distribution of the points. CO depresses the transport and subsequent liberation to tissues of O_2 by combining with Hb and as well as cyanide, it combines with cytochrome oxidase, the last step of the electron transport chain. CO combines with the reduced form of the enzyme and cyanide with oxidized form [7]. Under these circumstances, an additive action was at least expected to occur on simultaneous action of CO and HCN. However, this was not the case. The cause for the lack of the additive action remains unknown. The in vivo inhibitory effects of CO on the respira-

tory enzymes may not be great. By comparing the LC50 values for HCN of rodents with and without CO, Higgins et al [8] have reported that CO at the 25% COHb level had no effect on the acute toxicity of HCN. They have considered that the slightly decreased extracellular O_2 transport by CO did not significantly influence the action of HCN.

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