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THE TOXICITY OF FUMES FROM A DIESEL ENGINE UNDER FOUR DIFFERENT RUNNING CONDITIONS

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It has been suggested that fumes from diesel engines make an important contribution to atmospheric pollution. This is probably because they are sometimes highly irritant and sometimes very smoky. It is well known that, because of their comparatively low carbon monoxide content, these fumes are much less lethal than those from petrol engines.

The leading work on diesel fumes has been done by the U.S. Bureau of Mines. Holtz, Berger, Elliott, and Schrenk (1940) analysed the fumes from engines in proper mechanical conditions, plotting the carbon monoxide, nitrogen oxides, and aldehyde content against fuel-air ratio.

The present work was directed to ascertaining what were the main toxic constituents of diesel fumes. In order to do this animals were exposed to the fumes and at the same time the concentration of various constituents of the fumes was determined; pathological investigations were subsequently carried out on the exposed animals.

In this paper nitric oxide is designated NO, and nitrogen dioxide NO_2. For conversion of parts per million to mg./m³ factors valid at 20°C. have been used.

Apparatus and Materials

Engine.—The engine used was a Royal Enfield single cylinder air-cooled diesel engine. Detailed features of this engine are:—

Cylinder bore	3·346 in. (85 mm.).
Cylinder stroke	3·937 in. (100 mm.).
Cylinder capacity	568 ml.
Compression ratio	19·5 to 1.
Valve gear	Overhead operated by push rods.
Fuel injection	C.A.V. equipment operated from engine camshaft.
Atomizer	C.A.V. single hole type with "leak off" connexion.
Governor	Centrifugal type mounted on end of camshaft. Adjustable for any speed between 1,200 and 1,800 r.p.m.
Power developed	3·75 to 6·0 B.H.P. at 1,200 to 1,800 r.p.m.
Fuel consumption	0·42 pints/hr. (570 ml./hr.) at 1,500 r.p.m.

Fuel.—The fuel used was diesel fuel, 47 cetane, obtained from the Regent Oil Co. Ltd. It had been produced by distillation from Venezuelan mixed base crude. Its characteristics were as follows:—

Viscosity No. 1 Redwood at 100°F. (37·8°C.) (kinematic viscosity approx. 3·0 centistokes)	33 sec.
Specific gravity at 60°F. (15·6°C.)	0·838.
Flash point	210°F. (99°C.)
Neutralization number (total acidity)	0·1136 mg. KOH per gm. oil
Carbon (Conradson)	0·006%
Pour point	Below —30°F. (—34·4°C.)
Water content	Nil

Distillation range (10 mm. Hg)

	Initially	10%	20%	30%	40%	50%	60%	70%	80%	90%
°F	130°	184°	215°	235°	262°	283°	295°	321°	347°	371°
°C	54·5°	84·5°	102°	114°	128°	140°	146°	166°	175°	189°

Sulphur content 0·51% (determined by Institute of Petroleum lamp method)

Exposure Chamber.—The exit of the silencer of the engine was connected by a flexible metal pipe, 695 cm. long and 3·2 cm. in internal diameter, to a 10 cubic metre chamber. Two exhaust pipes from the chamber led to the outside air. The contents of the chamber were circulated by means of a fan. The temperature of the chamber during a run was 2-3°C. higher than that of the outside air. The walls of the chamber were of glass and painted steel; the floor was of concrete.

It is to be noted that in toxicity experiments with nitric oxide present the size of the chamber and the flow rate through it may have a considerable effect on the results. The reason is that the nitric oxide is slowly oxidized to the dioxide which is relatively more toxic. Hence if fumes of constant nitric oxide content are supplied to a chamber, the toxic effects on animals exposed in it will be greater the lower the rate of change of atmosphere in the chamber. In this case the fumes were supplied at a rate sufficient to fill the chamber in about 20 minutes (or 40 minutes under conditions D).

Experimental Methods

Running Conditions of Engine.—Four conditions of engine running were investigated. The essential features are given in Table 1.

Details of the running conditions are as follows:—

A. The engine was run with only its accessories,

including the cooling fan, taking power. Under these conditions the exhaust was acrid (causing lachrymation in under 10 seconds). At times it was almost clear, but sometimes white fumes were produced; the visibility in the chamber was then about 6 ft.

B. To the engine were coupled a large fan and two hydraulic pumps to provide a load. The fumes produced under these conditions were less acrid but more toxic than under a light load.

C. The load was arranged as under condition B but the original injector (which was in good mechanical condition) was replaced by one which was stated by the manufacturer to be " worn ". This replacement affected the fuel-air ratio but little; the acridness and toxicity of the fumes were, however, much diminished.

D. To produce a higher fuel-air ratio, the air inlet connexion was partly closed with a metal blank. The load consisted only of the accessories. The area covered was adjusted by experiment to give continuous white smoke from the exhaust. The open circular segment had an area of approximately 0·03 sq. in. (0·193 sq. cm.) as compared with the normal inlet area of 1·227 sq. in. (8·6 sq. cm.). Under these conditions the engine produced a dense white smoke, apparently consisting mainly of unburnt oil; very little carbon was present. The fumes were very acrid, causing intense pain to the eye in 4 to 7 seconds. The visibility in the chamber was only a few inches, and the lethal quality of the fumes was greater than under conditions A, B, and C. In the early stages of running under these conditions the firing was irregular, as if on some strokes the engine failed to fire; it grew more regular as the engine warmed up.

Sampling and Analysis.—To obtain an accurate assessment of the dosage to which the animals were exposed, all samples, other than that for total oxides of nitrogen, were collected at a constant rate throughout each experiment.

Samples of gas for CO and O_2 determination were taken by drawing fumes into a balloon at a uniform rate by means of a suction device. Carbon dioxide samples were collected by drawing the fumes through bubblers at 50 ml./min. by means of an aspirator. Other samples were collected in bubblers at 1 l./min., critical orifices being used to control the flow. To protect the orifices and balloons from dirt, the fumes were sucked through two Whatman No. 1 filter papers in series before entering the bubblers. The total particulate matter, black smoke, particulate acids, and vanadium contents of the samples collected on these papers were determined.

In every case two bubblers and two filter papers were used in series. Determinations were carried out on each separately, and the measured concentration was corrected for slip.

The fuel-air ratio was obtained by measuring the fuel consumption and (when the air intake was unobstructed) calculating the air intake from the cylinder volume and engine speed. A volumetric efficiency of 90% was assumed. Under conditions D the air intake was measured directly using a " rotameter " flowmeter.

Determinations of Oxygen, CO_2, and CO.—Oxygen was determined in a gas sample by Haldane's method, carbon dioxide by absorption in N/2 sodium hydroxide which was then titrated with acid using phenolphthalein and methyl orange as indicators, and carbon monoxide from a gas sample by means of the Grubb Parsons infra-red gas analyser.

Determination of Aldehydes.—Aldehydes were determined by absorption in hydroxylamine hydrochloride solution which was initially adjusted to *p*H 4·5. The aldehydes form oximes with the hydroxylamine, liberating hydrochloric acid, which is then titrated.

As the hydroxylamine hydrochloride has a buffering action, the concentration used must be chosen to suit the quantity of aldehyde to be determined; a large excess of the former must not be used. (Under conditions D insufficient hydroxylamine was used, and the concentration of aldehyde had to be determined in a subsidiary experiment.) This method is also sensitive to certain ketones, including acetone. Control bubblers containing distilled water were used to obtain a correction for acids directly absorbed from the fumes.

Total Particulate Matter.—This was determined by weighing the filter sample; the paper was previously weighed, a control filter paper being used as a counterweight to compensate for variations in atmospheric humidity.

Particulate Acids.—These were determined by titration of a filter sample at *p*H 4·5; they were expressed as sulphuric acid, but other acids such as formic and acetic may have been present.

Black Smoke.—Black smoke was determined by comparing the shade of the stain on the filters with those of a series of standard stains obtained from an atmosphere of smoke from a kerosene lamp. The concentration of smoke in this standard atmosphere was determined by weighing samples. As different smokes differ in blackening power, this method can give qualitative results only.

Vanadium and Nitrogen Dioxide.—It was shown by the method of Woodman and Cayvan (1901) that the concentration of vanadium in the fumes was less than 10 μg./m^3, if indeed it was present at all. The determination of nitrogen dioxide (NO_2) in the presence of nitric oxide was difficult. Holtz *et al.* (1940) determined only total oxides of nitrogen. Various methods were tried; values obtained by means of the optical absorptiometer of Sinkinson (1954) were found to be compatible with those obtained by absorption in alkali in two large sintered glass bubblers followed by determination of nitrite by the Griess-Ilosvay reaction. The mean of the values obtained by these two methods was used.

It was found that if a long train of bubblers is used oxidation of NO to NO_2 causes errors.

Total oxides of nitrogen were determined by admitting fumes to an evacuated bottle containing alkali. This was left overnight to allow complete oxidation of NO, and nitrite was determined by the Griess-Ilosvay reaction.

A colorimetric method for determining sulphur dioxide, based on its reaction with decolorized fuchsin, failed to give positive results owing probably to the presence of oxides of nitrogen. To obtain a value for the maximum possible sulphur dioxide content of the fumes under

conditions A a known volume of the fumes was bubbled through hydrogen peroxide (1 vol.), initially at *p*H 4·5, the acid produced being titrated.

Toxicity Experiments.—Preliminary experiments showed that the toxicity of the exhaust fumes varied somewhat from day to day even if no deliberate alteration to the running conditions was made. It was, therefore, necessary to repeat the whole series to see whether results reproducible enough to show any alteration in toxicity with running conditions could be obtained. In fact, while the mortalities under a given set of conditions were not always the same in the two series, the difference between them was much less marked than that between the mortalities under different running conditions. In both series the arrangement of running conditions in order of lethality was D, B, C, A. In Table 1 the mortalities in the two series are (where they differed) given separately.

It was found that animals could usually be exposed to undiluted diesel fumes for some hours without asphyxia.

Two series of experiments were performed. In each set eight rabbits, 20 guinea-pigs, and 80 mice were placed at random into four batches. Each batch was exposed in the chamber to undiluted diesel fumes for five hours or until all the animals were dead. Each of the four batches was exposed to fumes produced under one of the four running conditions described above. The whole experiment was then repeated using four batches from another set of animals. In Table 1 the results of these two sets are combined, but some individual experiments are referred to below as A1, A2, etc.

In an experiment, the engine was first started and left on for half an hour so that the chamber could fill with fumes. (The flow of fumes at room temperature was about 500 l./min. under conditions A, B, and C and about 250 l./min. under D.) The animals were then placed in the chamber in cages and sampling was started. At intervals the animals were inspected and any dead were removed. After five hours the engine was stopped and the chamber was cleared.

The next day, if there had been no mortality in any one of the species, one of the species was killed with nembutal and a post-mortem examination was performed.

Three weeks after each experiment on the first set, and one week after each experiment on the second set, all the surviving animals were killed with nembutal and examined at necropsy.

In all cases specimens of lung, trachea, and other tissues were taken from representative animals and sections were stained with haematoxylin and eosin. In cases where carbon monoxide poisoning was suspected (conditions D) carboxyhaemoglobin was determined at necropsy by colour comparison using a Lovibond's comparator.

Lachrymation time was measured by an observer applying his eye to a port in the chamber wall and noting the time required to produce intolerable irritation.

Results

The mortality data and the concentrations of the more significant components are summarized in Table 1. This gives the mean of all the reliable determinations made under given running conditions. The total mortalities are also summarized. The less important constituents are discussed below.

The oxygen concentration was above 16% in all cases except in D2, when it was 11·4%. Carbon dioxide ranged from 2·34% to 3·58%. Particulate acids were equivalent to less than 1 p.p.m. of sulphuric acid in all cases. The maximum black smoke content found was 2·2 mg./m³ (conditions C), but this determination is, as explained, qualitative only. Gaseous acids were found equivalent to 17 p.p.m. of sulphur dioxide at the exit of the silencer, and 5·8 p.p.m. in the chamber. The vanadium content of the fumes was less than 10μg./m³.

The following points not given in Table 1 are worthy of record:—

In no case did the fumes appear black or smoky. The liquid collected from them appeared to be unburnt diesel oil. A smoky exhaust, such as is frequently criticized as a source of atmospheric pollution, was not used in this series of experiments.

The subjective differences in the irritancy and lachrymating power were greater than might appear from the measured lachrymation times. Under condition D the fumes were violently irritant, and produced severe and lasting pain in the eyes. The change in irritancy on replacing the "good" injector (B) by a "worn" one (C) was very marked. When the latter was used the fumes were almost non-irritant. Likewise the fumes under condition A were definitely more irritant (though less toxic) than those under condition B.

There are significant differences between the mortalities recorded with the same conditions and animals from different batches. In conditions B and C, deaths usually took place in the last hour of the five-hour exposure, or on the following day. In conditions D, the mice died first; they died more rapidly in D1 than in D2. After one hour's exposure in D1 (0·22% CO), all except three of the 20 mice were dead. All the rabbits and guinea-pigs were alive. After 3 hr. 20 min. all the animals were dead and the experiment was stopped. In experiment D2 (0·12% CO), on the other hand, one mouse only was dead at the end of an hour. After 3 hr. 20 min. 19 mice and one guinea-pig were dead, but both rabbits were still alive. After 4 hr. 35 min. all the animals were dead.

Post-mortem Findings: Macroscopic.—One of each from the animals exposed under conditions A was killed with nembutal the day after exposure. In general the appearance of the lungs was normal but in one mouse there was congestion of a hepatic type. As there were no fatalities in this group it seems

TABLE 1

SUMMARY OF EXPERIMENTS ON EXPOSURE OF ANIMALS TO DIESEL FUMES

Running Conditions	A	B	C	D
Speed (r.p.m.)	1,600	1,600	1,600	1,600
Power output (kw.)	Low	4·1	4·1	Low
(B.H.P.)	Low	5·5	5·5	Low
Fuel-air ratio	0·0121	0·0193	0·0199	0·0333
Injector condition	Good Open	Good Open	Worn Open	Good Restricted
Composition and Properties				
CO (%)	0·056	0·041	0·038	0·17
NO_2 (p.p.m.)	23	51	43	12
Oxides of N (as NO_2) (p.p.m.)	46	209	174	44
Aldehydes (as formaldehyde) (p.p.m.)	16	6·0	6·4	154(*c*)
Particulates (mg./m³)	74	122	53	1,070
Visibility (ft.)	6	1 to 6	6	0·5
Lachrymation time (sec.)	7·5	10	20	5·5
Animals				
Duration of exposure (hr.)	5	5	5	5
Mortality (*a*) during exposure (*b*) total (up to 7 days)				
Out of 40 mice	(*a*) 0 (*b*) 0	(*a*) 13 (*b*) 19 (*d*) 10;3 (*d*) 13;6	(*a*) 0 (*b*) 1 (*d*) 0;0 (*d*) 1;0	(*a*) 40 (*b*) 40
,, ,, 10 guinea-pigs	(*a*) 0 (*b*) 0	(*a*) 0 (*b*) 9 (*d*) 0;0 (*d*) 4;5	(*a*) 1 (*b*) 6 (*d*) 0;1 (*d*) 1;5	(*a*) 10 (*b*) 10
,, ,, 4 rabbits	(*a*) 0 (*b*) 0	(*a*) 0 (*b*) 0	(*a*) 0 (*b*) 0	(*a*) 4 (*b*) 4
Pathology				
Tracheal damage	Mild	Nil	Nil	Nil (mice) Severe (others)
Lung damage	Mild	Severe	Moderate	Nil (mice) Mild (others)
Carboxyhaemoglobin	Low	Low	Low	60% (mice) 50% (others)
Most sensitive animal	Guinea-pig	Guinea-pig	Guinea-pig	Mouse
Postulated main cause of death:				
Mice	(1) Irritants (*e*) (2) NO_2 (*e*)	NO_2	NO_2	CO
Guinea-pigs	(1) Irritants (*e*) (2) NO_2 (*e*)	NO_2	NO_2	(1) CO (2) Irritants
Rabbits	(1) Irritants (*e*) (2) NO_2 (*e*)	—	—	(1) CO (2) Irritants

(*c*) Determined in a subsidiary run
(*d*) Mortalities in the two series are given below the total mortality in each case
(*e*) In exposures of seven or 14 hours

likely that this mouse would have recovered. Animals killed one or three weeks after exposure under conditions A sometimes showed emphysematous raised areas or patches of congestion in the lungs but were usually normal in their post-mortem appearance.

In the animals which died after exposure under conditions B, there was severe pulmonary damage. The greatest damage was found in those animals which survived the exposure and died the next day. Nearly always there was severe oedema with frothy fluid oozing from the cut surfaces of the lung. Often there were alternating patchy areas of consolidation and oedema. In many cases froth oozed from the trachea. The blood was normal in colour, even in animals dying in the fumes. The carboxyhaemoglobin content was low. The stomachs of the guinea-pigs were normal.

Similar findings were made in animals dying under conditions C; in some guinea-pigs alternating patches of consolidation, congestion, and emphysema gave the lungs a " tortoiseshell " appearance.

The lungs of animals surviving exposures under B and C conditions, and killed one or three weeks later, sometimes showed evidence of previous injury. In two mice killed three weeks later there were sharply delimited patches of consolidation at the base of the lung. Another mouse had widespread congestion of the lungs; another had complete consolidation of the right lung. In many cases, however, the lungs of survivors were of normal appearance.

The blood of the animals which had died under conditions D was scarlet. The carboxyhaemoglobin content in the mice was (by Lovibond's method) about 60%, and in the other animals about 50%.

The lungs of the mice which had died under conditions D were of normal appearance.

The guinea-pigs and rabbits which died under these conditions showed varying amounts of emphysema and congestion. One rabbit showed oedema. All the guinea-pigs showed petechial and confluent haemorrhages of the stomach with desquamation of the mucosa, and one of the rabbits showed scattered discrete small haemorrhages in the cardiac region of the stomach. One of the rabbits showed many small haemorrhages of the thymus.

Post-mortem Findings: Microscopic.—Those animals which died during the exposures all showed some degree of pulmonary abnormality. Moderate to intense capillary and venous congestion were constant findings. In the mice which had died under conditions D, the lung damage consisted mainly of intense congestion and small capillary haemorrhages, probably pre-terminal.

The degree of lung damage was rather greater in the guinea-pigs and rabbits from the D exposures than in the mice.

Animals which died during the B and C exposures all showed more severe lung damage. Oedema was a fairly constant feature, more marked in the guinea-pigs than in the mice; of the latter a few showed traces only of oedema. In those cases where consolidation was present this was invariably associated with alveolar collapse and oedema. Emphysema was also present. In the mice, damage to the bronchial and bronchiolar epithelium was slight, whereas the guinea-pigs showed commencing bronchiolitis.

Animals which died after removal from the fumes invariably showed much more severe lung damage than those which died during the experiment. This is to be expected, as not only was exposure longer for the survivors but the delayed effects of the nitrous fumes had more time to develop. In the guinea-pigs in particular there was severe oedema.

Animals killed the day after the experiment (because no member of their species had died) showed little damage. Mice exposed under conditions A had slight oedema but in general the lungs were almost normal. Guinea-pigs had some patchy consolidation of the lungs with some emphysema. There was rather more damage in experiment A2 (22 p.p.m. aldehyde) than A1 (9·9 p.p.m. aldehyde).

The animals killed seven or 21 days after the experiment showed varying stages of repair.

The tracheas of the animals in experiments B and C showed little damage, and the same was true of the larger bronchi. Slight damage was evident in experiment A; a rabbit exposed in A1 and sacrificed the next day also showed desquamation of the epithelium of the trachea.

The larger bronchi of the mice in experiment D showed no damage, but the tracheas and bronchi of the guinea-pigs and rabbits in experiment D showed very serious damage indeed. In one guinea-pig the whole of the tracheal epithelium had been removed and there was evidence of formation of a false membrane. In one rabbit the thymus was found to be haemorrhagic. The abnormality appeared to be vascular engorgement with many small capillary haemorrhages.

Subsidiary Experiments

Acrolein.—Acrolein was used to determine the pathological effects of a typical organic irritant.

Two rabbits (2·6 and 2·8 kg.), four guinea-pigs (mean weight 228 g.), and 20 mice (mean weight 20 g.) were exposed for six hours to acrolein at a mean concentration of 10·5 p.p.m. (24·4 mg./m^3). The total dosage was 8,790 mg.min./m^3. At the close of the experiment all the animals were alive; the mice were in a very somnolent condition, as if they had had an insufficient dose of anaesthetic. One guinea-pig and two mice were killed for post-mortem examination: another guinea-pig died shortly afterwards and another overnight. Eight mice died the next day, and a further mouse the day after. The mortality was 1/2 for rabbits, 2/4 for guinea-pigs, and 9/20 for mice.

In the one guinea-pig and two mice killed immediately after the exposure there was macroscopically little evidence of abnormality. A clear frothy fluid was present in the guinea-pig's trachea. Sections of the lungs showed slight changes only; there was acute emphysema and in the guinea-pig patchy areas of oedema. The epithelium of the bronchi and bronchioles was intact and showed slight increase in its mucous covering.

The guinea-pig which died shortly after exposure had lungs which were voluminous but of almost normal appearance. Sections showed widespread emphysema with some partial consolidation, oedema, and congestion. Slight inflammatory cell infiltration was present. Another guinea-pig dying overnight had both upper lobes of the lungs completely consolidated. Microscopically there were acute emphysema, widespread oedema, and generalized capillary congestion. The trachea showed severe damage with much desquamation of the epithelium. In the lumen was found almost non-cellular, partly fibrinous, exudate. There was a moderate degree of inflammatory cell reaction; the picture was one of acute desquamating tracheitis.

Mice dying the day after exposure showed gross pulmonary changes. In the trachea of one of them the epithelium had been replaced by a cellular exudate, mainly of inflammatory blood cells. In the lungs there were consolidation, congestion, and inflammatory cell reaction. In the rabbit which died 36 hours after exposure, there was found generalized emphysema with patchy areas of congestion and consolidation. The epithelium of the bronchi had desquamated and some bronchi were partly plugged.

The picture of acrolein poisoning is, therefore, one of gross damage to the trachea, bronchi, and lungs. This contrasts with the action of diesel

fumes under conditions B and C, where damage to the trachea was slight. Deaths which were delayed for more than 24 hours after exposure occurred with acrolein, but not with diesel fumes.

Diesel Fumes (Long Exposures under Conditions A).—As five hours' exposure produced no fatalities, the results of two long exposures are reported.

Two rabbits, 10 guinea-pigs, and 50 mice were exposed to undiluted fumes from the engine running under conditions A (light load). On the first day the exposure time was seven hours; nine of the guinea-pigs died.

Overnight the remaining guinea-pig, one rabbit, and 10 mice died.

The next day exposure continued for a further seven hours; the remaining rabbit and 35 of the mice died during exposure.

The mortalities for the three species were therefore:—

7-hour exposure	1/2, 10/10, 10/50
14-hour exposure	2/2, 10/10, 45/50

Within half an hour of the beginning of the experiment the mice became lethargic. The behaviour of the other animals was normal.

No chemical analysis was performed during this experiment.

Macroscopically the lungs of animals which had died during or after exposure for seven or 14 hours to fumes under conditions A showed varying degrees of pulmonary congestion, consolidation, oedema, and emphysema. In guinea-pigs which had died, a frequent finding consisted of small, widespread, well-defined areas of emphysema alternately with depressed areas of consolidation. Other organs showed no constant definite abnormality, though the livers were uniformly pale and in two rabbits were beginning to show a nutmeg appearance.

Microscopically in all the animals examined there was a moderate to severe degree of tracheitis; the epithelium in parts was missing with very few basal cells remaining. Many of the remaining epithelial cells were faintly stained. Epithelium where missing was invariably replaced by a layer of densely cellular exudate, the cells being mainly polymorphs. Inflammatory cells infiltrated the submucosa and (infrequently) the cartilaginous ring. Microscopic examination of the lung sections confirmed the macroscopic findings. The constant striking features were patchy areas of emphysema and partial alveolar collapse associated with oedema and capillary congestion. Haemorrhages were not a constant finding. In all sections there was seen an acute inflammation of the entire respiratory tract. Many of the bronchi and bronchioles were plugged with cellular exudate, like that in the trachea.

One rabbit and two guinea-pigs showed early bronchopneumonia.

In the mice, the pulmonary changes were not as constant as those seen in the guinea-pigs. The epithelium of the bronchi and bronchioles was intact and inflammation, where present, was of a lesser degree than in the guinea-pigs and rabbits.

The picture was, therefore, that of gross damage to all parts of the respiratory tract.

Discussion

Composition of Fumes.—The most obvious finding concerning the composition of the fumes was that in different trials under the same running conditions the composition was different. That these variations are genuine is confirmed by the fact that in experiment D2 (with 0·12% CO) the mice took much longer to die than in experiment D1 (0·22% CO). The differences between experiments under the same running conditions are, however, less than those found under different running conditions.

Holtz *et al.* (1940), in their work on the composition of diesel fumes, adopt the " fuel-air ratio " as a standard of comparison, and we have followed their example. The " chemically correct " fuel-air ratio, at which there is just enough oxygen for combustion of the fuel, is about 0·068. In our experiments, the fuel-air ratios from 0·0119 to 0·036 were explored. The highly abnormal conditions D (air intake obstructed) were not used by Holtz *et al.*

Oxides of Nitrogen.—Holtz *et al.* (1940) found that for a given engine the total oxides of nitrogen (except N_2O) had a maximum concentration, equivalent to 340 to 590 p.p.m. (650 to 1,250 mg./m³) NO_2, at intermediate values of fuel-air ratio (0·025 to 0·045); the concentration with low fuel-air ratio (0·010 to 0·015) was less (50 to 250 p.p.m., or 96 to 480 mg./m³). This is in accordance with our finding more oxides of nitrogen under moderate load (B) than under light load (A). With the worn injector, we found less oxides of nitrogen. These workers did not determine separately the concentration of nitrogen dioxide.

Hanson and Egerton (1937) found that the ratio of NO_2 to total nitrogen oxides in the exhausts of diesel engine was higher with no load than on load; our findings are in accord.

Aldehydes.—Holtz *et al.* (1940) found that the concentration of aldehydes in the fumes decreased as the fuel-air ratio increased. This is in accord with our findings when experiments A are compared with B and C. D covers conditions not used by Holtz *et al.* It must be emphasized that the method used by us for determining " aldehydes " also determines at least some ketonic bodies, and this may be true

of other methods also. They found (Holtz *et al.* 1943) that aldehydes were higher with a " leaking " injection valve; but inspection of their tables shows that installing a valve with " lopsided " or " sticky " sprays (the latter giving a spray the angle of whose cone was less than normal) caused a reduction in the aldehyde content. The effect on aldehyde content of installing our worn injector was irregular, although the reductions in irritancy and toxicity were very marked.

Carbon Monoxide.—Our findings under conditions A, B, and C are in accord with those of Holtz *et al.* (1940) who found a minimum CO content at a fuel-air ratio of about 0·03.

Black Smoke.—Holtz *et al.* did no direct measurements of the amount of soot in the exhaust, but found smoky exhausts at fuel-air ratios above the chemically correct ratio. Very little black smoke was produced in our experiments, even under conditions D. Nearly all the particulate matter found in our experiments was of an oily nature and was not of dirty appearance.

Vanadium.—Browne (1955) states that the exhausts of gas turbines using residual oils contain 20-60 p.p.m. by weight of vanadium compounds, equivalent to perhaps 6-18 mg./m³ of vanadium. The concentration in the exhaust of our engine was less than 1/500 of this. Sjöberg (1950) found that rabbits exposed to 20-40 mg./m³ of vanadium pentoxide dust for one hour a day for eight months developed emphysema; the concentration of vanadium in our experiments was therefore a long way below the toxic level.

Browne (1955) states that the vanadium content of residual oil from Venezuela (from whence the fuel for our experiments was derived) is higher than that of other residual oils. The low vanadium content of the fumes in our experiments was probably therefore due to our fuel being distillate and not residual oil.

Sulphur Oxides.—There is a discrepancy between the amount of sulphur found in the fumes and that supplied in the fuel. With fuel of composition 84% C, 0·51% S, the fumes should contain 23 p.p.m. of sulphur dioxide or sulphuric acid for each 1% of carbon dioxide plus carbon monoxide. Our fumes contained very little sulphuric acid mist; with a carbon oxides content of 2·4% (conditions A) there should have been 55 p.p.m. of SO_2, while the measured content was 17 p.p.m. It is possible that some of the sulphur may appear in the fumes as an organic compund of sulphur, as elementary sulphur, or as carbon oxysulphide. Berger, Elliot, Holtz, and Schrenk (1943) found that the amount of sulphur in the fumes from their engine was equal to that in the fuel consumed; the discrepancy in our results is unexplained.

Irritancy.—The fumes produced in experiments D were exceptionally irritant, those in experiments A highly so. The fumes of B were irritant, while the irritation from C was only slight. These differences are borne out by the measured lachrymation times. The irritancies are roughly in line with the aldehyde contents except when B is compared with C. It has been found, however (Sim and Pattle, 1955), that aldehydes differ greatly among themselves in irritancy, acrolein and crotonic aldehyde being at least a hundred times as irritant as the corresponding saturated compounds, propionic and butyric aldehydes. Again, the fumes in experiment C2 (aldehyde content 8·3 p.p.m.) were much less irritant than those in experiment B2 (4·1 p.p.m. aldehyde). Chemical composition, as expressed in terms of broad groups of substances is, therefore, unreliable as a guide to the irritant properties of the fumes. These properties can be affected by quite subtle differences in combustion conditions, and the fact that our least irritant fumes were obtained with a faulty injector demonstrates the fallacy of supposing, as does Turner (1955), that the requirements for mechanical perfection in an engine are identical with those for a physiologically or medically harmless exhaust.

The exact nature of the irritant or irritants present cannot at the moment be stated. The known irritancy of acrolein, which caused severe discomfort at 1·2 p.p.m. (Sim and Pattle, 1955), suggests that the presence of a small amount of acrolein among the aldehydes would cause the observed effects. Bodies reacting as aldehydes, but less irritant than acrolein must, however, also be present. The irritancy of fumes containing 22 p.p.m. of aldehydes, etc., was considerably greater than that found by Sim and Pattle for 20 p.p.m. of formaldehyde. If the irritation is due to aldehydes, therefore, bodies more irritant than formaldehyde must be present.

Toxicity.—In considering which of the substances found in the diesel exhaust fumes had toxic effects the simplest case is that of the mice in experiment D. Here the high carboxyhaemoglobin value in the blood, and the virtual absence of lung damage (other than congestion, which may be caused by heart failure) point to carbon monoxide poisoning as the cause of death.

The other animals in experiment D were not free from damage to the trachea, and this may have been due to their longer survival in the fumes. In

these cases the concentration of carbon monoxide was such that the animals would probably have succumbed even in the absence of the irritant bodies; the latter may have accelerated death. Acrolein administered alone produced tracheal damage, and this or some similar body could have caused it in these cases. Nitrogen dioxide was probably not important.

The fumes in B and C were much less irritant than in A, and contained less aldehyde; but they were far more toxic than those in A. Tracheal damage was negligible. It is, therefore, likely that irritant aldehydes played little part in causing death. The high concentration of nitrous fumes and the gross oedema of the lungs found (especially when death was delayed) point to nitrogen dioxide as the cause of death in these cases. The lower toxicity in C (worn injector) must be attributed to a lower concentration of NO_2 rather than to absence or irritant aldehydes.

It may be supposed that in B and C the carbon monoxide accelerated death, and that in its absence survival would have been longer and lung damage greater than was actually observed. Bearing this in mind our results are in accord with those of La Towsky, MacQuiddy, and Tollman (1941) who found 55 p.p.m. of NO_2 for two to three hours, a lethal exposure in some cases.

In the main experiments five hours' exposure under conditions A caused no fatalities. There was some damage both to the trachea and to the lungs. To assess the main toxic hazard under these conditions we must turn to the results of the subsidiary experiments, in which animals were exposed for seven hours or more. In general there was in those animals dying from exposure to the fumes under conditions A severe damage to the whole of the respiratory tract, including tracheitis and pulmonary oedema. This suggests that irritants of the acrolein type, and probably also the nitrogen dioxide which was present, played a part in causing the deaths and that under these conditions they both constituted toxic hazards.

In all cases NO was present in greater quantity than NO_2. The toxicity of NO is somewhat similar to that of carbon monoxide (0·25% is lethal in 12 min.) (Flury and Zernik, 1931). It combines (as does CO) with haemoglobin, and probably reinforced the action of the CO which was present. Its effects are quite different from those of NO_2, and it cannot be considered a major toxic constituent of the fumes.

The somnolent behaviour of the mice in these experiments was probably caused by organic bodies having anaesthetic properties; this action may in some measure protect the mice from the toxic gases.

Conclusion

The conclusion reached as the result of these experiments is that the toxic substances in diesel exhaust fumes and their relative importance vary according to the working conditions of the engine.

With the engine and fuel used it appears that the chief toxic substances are:—(1) Under light load, irritants of the type of acrolein and, to a less extent, nitrogen dioxide, (2) under moderate load, nitrogen dioxide (NO_2), (3) under light load with restricted air intake, carbon monoxide and to a less extent irritants.

General Remarks

Further Work.—It has been shown that under some conditions the irritancy and the toxicity of diesel fumes are due to entirely different factors. The determination of two of the main toxic factors—nitrogen dioxide and carbon monoxide—is quite possible, though in the former case far from easy. Identification, and still more determination, of the substance or substances responsible for eye irritation and tracheal damage seem to be very difficult. Determination of groups of substances, such as aldehydes, can give only a general idea of how much irritant might be present. Attempts to deduce the irritant or toxic action of the fumes from their measured composition are, therefore, very liable to error. Comparative measurements of lachrymation time, or subjective estimates of the relative irritancy of fumes under different running conditions, are easy to make. It is, therefore, highly desirable that anyone working on, for instance, the variation of composition of the fumes with running conditions, should at the same time test irritant and lachrymatory properties of the fumes, and if possible investigate their toxicity. Our experiments B and C show the great change in irritancy which can occur without marked change in the chemical findings.

In general it must be emphasized that as many measurements as possible of engine running conditions, fume composition, and irritancy should be made at the same time. Argument from one engine to another, from one worker's analysis to another's, or even from one day's performance by an engine to the next day's, is liable to fall into error.

Nitrogen dioxide is formed in large quantities in the petrol engine (Hanson and Egerton, 1937). There is, therefore, no reason to suppose that the toxicity of the fumes from the petrol engine, even if it were not masked by their much greater carbon monoxide content, would be less than that of diesel fumes; they might however have less effect on the trachea and bronchi than do diesel fumes. The main objection to diesel fumes seems to be their irritant nature.

Summary

Experiments have been carried out to ascertain what are the main toxic constituents of diesel fumes.

A single cylinder diesel engine was run under four different conditions: light load, moderate load, moderate load with a worn injector, and light load with the air intake restricted.

Mice, guinea-pigs, and rabbits were exposed to the undiluted fumes and at the same time determinations of a number of constituents of the fumes were carried out. Pathological investigations were carried out on the exposed animals. Animals were also exposed to acrolein vapour to assess its toxicity and mode of action.

The results of the analysis of the fumes were in broad agreement with those of other workers, except in regard to the sulphur oxides content; the latter was unexpectedly low.

Under light load the engine produced highly acrid fumes. Five hours' exposure caused no deaths, but seven hours' exposure did so. It is concluded that organic irritants (possibly aldehydes) and also nitrogen dioxide were the main toxic constituents.

Under moderate load, the engine produced fumes which were less acrid but more lethal, a high proportion of the animals dying during or shortly after exposure. Nitrogen dioxide is considered to have been the main toxic agent.

Replacement of a sound injector by one said to be " worn " made the fumes much less acrid and also less lethal. The lower toxicity is considered to be due to a lower nitrogen dioxide content.

When the air intake of the engine was restricted, very acrid fumes with much oily particulate matter were produced. This fume was lethal to all animals exposed for five hours. Carbon monoxide was the chief toxic constituent.

Under the running conditions used very little black smoke was present in the exhaust.

The procedure to be followed in future research is discussed.

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