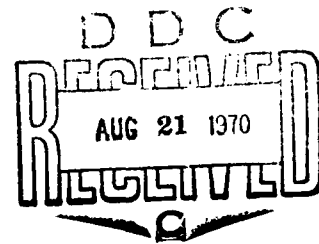


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ALIEN CONSTITUENTS IN EXPERIMENTAL ATMOSPHERES

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INTRODUCTION

Despite increased emphasis on inhalation toxicology in recent years it has not seemed standard practice to monitor experimental atmospheres for unexpected or unintended constituents. However, composition of atmospheres for animal exposure studies, even in simple design systems, may be quite different from expected. While little has been reported in this area in toxicologic literature, it should be recognized that physical and/or chemical interactions may occur in gas, liquid, or solid phase among pollutants at any point in the exposure system (e. g., generation, distribution, exposure chamber), and between pollutants or products and the animals themselves in the chamber, resulting in undesirable effects on the primary study agent(s) and/or formation of new and alien gaseous or particulate constituents.

This report concerns examples of the actual occurrence of just such phenomena, some details of identification and quantitation of components of a resultant alien particulate, and mention of interest and speculation which arises concerning the influence of such alien material with respect to effects on the study subjects, study design and interpretation, and the power of extrapolation from the experimental exposure to a realistic exposure situation.

MATERIALS AND METHODS

Nitrogen Dioxide Study

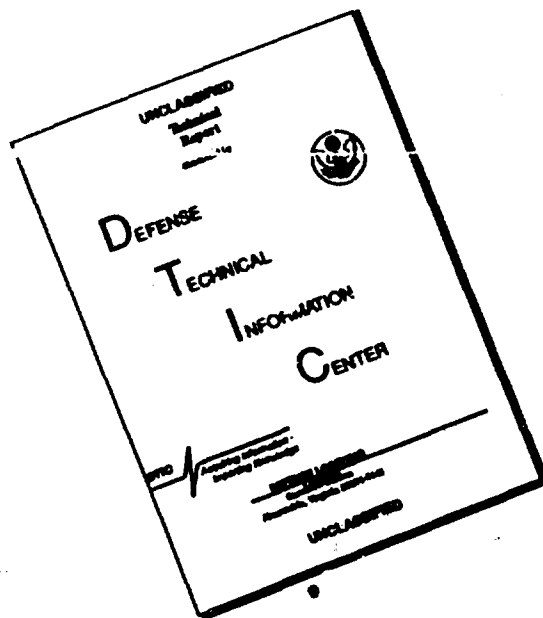
During a study involving exposure of dogs to an atmosphere containing relatively high concentrations of nitrogen dioxide (NO<sub>2</sub>) and ferric oxide particulate (Fe<sub>2</sub>O<sub>3</sub>), the presence of unintentional particulate material was detected.

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Exposure chambers, described by Hinners et al (1968), were of approximately  $2.83 \text{ m}^3$  (100 ft.<sup>3</sup>) capacity in the animal exposure portion and were constructed of glass and stainless steel with cubical dimensions of  $1.5 \times 1.5 \times 1.2 \text{ m}$  (5 x 5 x 4 ft.) and conical top and bottom. Chamber construction features provided a turbulent-mixing air flow pattern. Each was ventilated with a purified (CBR chemical and particulate-filtered), conditioned (70-75 F, 40-60% RH) air supply at the rate of  $1.4 \text{ m}^3/\text{min}$  (50 cfm), resulting in an atmosphere exchange rate of 30 per hour. Eight female pure-bred beagle dogs, each weighing approximately 11.3 kg (25 lbs) were exposed in each chamber.  $\text{NO}_2$ , generated by mixing dry filtered air with effluent from heated cylinders of 100%  $\text{NO}_2\text{-N}_2\text{O}_4$ , and  $\text{Fe}_2\text{O}_3$  aerosol with a mass median diameter of  $0.54 \mu$  generated from commercial powdered  $\text{Fe}_2\text{O}_3$  in a device described by Crider et al (1968), were introduced at the chamber air inlet duct at a rate to produce concentrations of 20 to 30 ppm  $\text{NO}_2$  and approximately  $0.8$  to  $1.0 \text{ mg}/\text{m}^3$   $\text{Fe}_2\text{O}_3$  in the chamber at exposure level. The animal exposure pattern was established at about 22 hours per day, 7 days per week, for 6 to 8 months. Chamber atmospheres were assayed for  $\text{NO}_2$  by the method of Saltzman (1954) and monitored for  $\text{Fe}_2\text{O}_3$  aerosol by a Sinclair-Phoenix Forward Scattering Aerosol Analyzer, \*\* and flame emission aerosol monitor (Crider et al, 1968).

It was noticed during particulate monitoring in the initial phase of the study that the combined presence of dogs and  $\text{NO}_2$  contributed substantially to the aerosol loading in the chamber. Figure 1 shows the Sinclair-Phoenix response to the  $\text{NO}_2$  and dogs in a clean chamber. This extraneous aerosol response was more than twice that which represented the  $\text{Fe}_2\text{O}_3$  concentration to be used in the toxicity study.

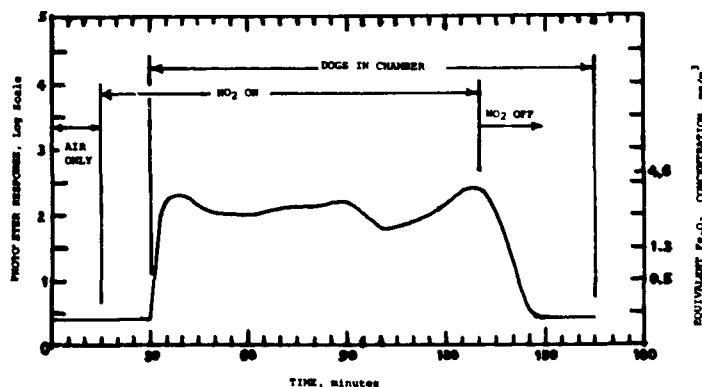


Figure 1. AEROSOL PHOTOMETER RESPONSE TO CHAMBER ATMOSPHERE ( $\text{Fe}_2\text{O}_3$  OFF)

\*\*Mention of commercial products does not necessarily constitute endorsement by the USPHS or DHEW.

In subsequent investigations of this phenomenon three factors were considered (presence of dogs, nitrogen dioxide, and  $\text{Fe}_2\text{O}_3$ ), combinations of which resulted in 8 chamber conditions: 1) no dogs or pollutants, filtered air supply only (designated O); 2) dogs alone (D); 3)  $\text{NO}_2$  alone (N); 4)  $\text{Fe}_2\text{O}_3$  alone (F); 5) dogs +  $\text{NO}_2$  (DN); 6) dogs +  $\text{Fe}_2\text{O}_3$  (DF); 7)  $\text{NO}_2$  +  $\text{Fe}_2\text{O}_3$  (FN); 8) dogs +  $\text{NO}_2$  +  $\text{Fe}_2\text{O}_3$  (DFN). To minimize interruption of the toxicity study in progress, conditions were established from a fully operating chamber by removing the dogs and/or discontinuing generation of  $\text{NO}_2$  and/or  $\text{Fe}_2\text{O}_3$ . Time of removal was designated immediately as zero, beginning from which for each condition six consecutive 30 minute aerosol samples were collected from the chamber by constant-rate (7.4 lpm) pumping through acid washed glass fiber filters. Weight, pH, conductivity determinations and chemical analyses for ammonium, nitrate and nitrite were performed on all samples as described elsewhere (Knott and Malanchuk, 1969).

### Ozone Study

During a study to assess the inhalation toxicity of ozone ( $\text{O}_3$ ), and employing the same chambers (Hinners et al, 1968), particulate counts from chambers were made under similar combinations of critical factors (with and without dogs and/or ozone) while controlling other potentially influential factors. The objective was to assess the particulate situation in relation to presence of dogs, ozone or both, compared to an empty chamber, with respect to enumeration and size distribution. In this work the particulate sampling, counting, and sizing was accomplished by use of an electronic particle counter. \*\* The study subjects were 8 female purebred beagle dogs, as in the previous ( $\text{NO}_2$ ) study. Ozone was generated from purified oxygen and introduced to the filtered, purified chamber air supply inlet so as to result in chamber concentrations in the range of 0.8 to 1.3 ppm. Chamber  $\text{O}_3$  concentrations were monitored by a Mast meter\* and by periodic wet chemical assay (neutral buffered KI), but no chemical characterization of aerosol was attempted.

### $\text{NO}_2/\text{O}_3$ Studies

In other work employing similar but smaller (approximately 0.34 m<sup>3</sup>) steel-glass animal chambers (Hinners et al, 1968) concentrations of  $\text{O}_3$  and  $\text{NO}_2$ , when introduced singly and in combination, were determined for establishment of standard operating conditions to be used in animal exposure studies. While the  $\text{NO}_2$  -  $\text{O}_3$  assays did not involve animals in the chamber or aerosol collections/assays, interesting effects on the  $\text{O}_3$  and  $\text{NO}_2$  were demonstrated (Ulmer, 1967).

\*\*Royco Instruments, Inc., Model 220

\*Mast Development Company

## RESULTS

Nitrogen Dioxide Study

In all analyses of samples of significant quantity, nitrate and ammonium ions were relatively abundant, while nitrite salts were absent. Compared to a reference curve (condition 0) of components, prepared by removing all generation and dogs at time zero and then sampling for 3 hours, curves of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  indicated the immediate beginning of chamber clearance so that the 30 to 180 and 150 to 180 minute sampling periods reasonably represented the equilibrium state resulting from removal of factors D, F, and/or N. Presence of dogs resulted in more rapid removal of the foreign aerosol material, indicated by measured  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , following discontinuation of  $\text{NO}_2$ ; i. e., these components decreased more abruptly when dogs were present, and at equilibrium (at end of sampling) were closer to zero in the FD and D conditions than in F and O, respectively.

When the chamber was receiving  $\text{NO}_2$ , the presence of dogs and  $\text{Fe}_2\text{O}_3$  gave approximately additive results, in terms of the ammonium nitrogen ( $\text{NH}_4 - \text{N}$ ) and nitrate nitrogen ( $\text{NO}_3 - \text{N}$ ) components of collected aerosol. This is suggested by the fact that, on a weight/volume basis,  $\text{NO}_3 - \text{N}_{\text{DN}} \approx \text{NO}_3 - \text{N}_{\text{FN}}$ ,  $\text{NH}_4 - \text{N}_{\text{DN}} \approx \text{NH}_4 - \text{N}_{\text{FN}}$ ,  $\text{NO}_3 -$

$\text{N}_{\text{DN}} + \text{NO}_3 - \text{N}_{\text{FN}}$ ,  $\text{NO}_3 - \text{N}_{\text{DFN}}$ , and  $\text{NH}_4 - \text{N}_{\text{DN}} + \text{NH}_4 - \text{N}_{\text{FN}}$ ,  $\text{NH}_4 - \text{N}_{\text{DFN}}$ ; also,

$(\text{NH}_4 + \text{NO}_3)_{\text{DN}} \approx (\text{NH}_4 + \text{NO}_3)_{\text{FN}}$  and  $(\text{NH}_4 + \text{NO}_3)_{\text{DN}} + (\text{NH}_4 + \text{NO}_3)_{\text{FN}} \approx (\text{NH}_4 + \text{NO}_3)_{\text{DFN}}$ .

Very little  $\text{NH}_4^+$  and  $\text{NO}_3^-$  was formed when  $\text{NO}_2$  was generated in the absence of both dogs and  $\text{Fe}_2\text{O}_3$ .

Based on sample averages over the 30-180 minute periods, the greatest absolute amounts of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were formed in the DFN, DN, and FN conditions, especially DFN; considerably less in the N and F conditions; and very little in the D, DF, and O conditions. Similarly, in terms of percent of total aerosol accounted for by assayed  $\text{NH}_4^+ + \text{NO}_3^-$ , the greatest proportion of aerosol accounted for by  $\text{NH}_4^+ + \text{NO}_3^-$  occurred in the DFN, DN, and FN conditions, especially DN.

Table I summarizes the average composition of chamber atmospheric particulate samples under the various conditions studied.

TABLE I  
 AVERAGE COMPOSITION OF AEROSOL SAMPLES WITH  
 VARIOUS CHAMBER CONDITIONS

Condition	Total Samples	NH <sub>4</sub> - N	NO <sub>3</sub> - N	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup> + NO <sub>3</sub> <sup>-</sup>
	$\mu\text{g}/\text{m}^3$	$\mu\text{g}/\text{m}^3$	$\mu\text{g}/\text{m}^3$	% of Total	% of Total	% of Total
(1)	(2)	(3)	(3)	(4)	(4)	(4)
DFN	1962	$\frac{108.4}{98}$	$\frac{183.3}{150}$	5.7	34.4	40.1
DN	657	$\frac{51.2}{52}$	$\frac{106.0}{107}$	7.6	54.2	61.8
FN	1215	$\frac{39.4}{45}$	$\frac{86.4}{88}$	4.1	31.0	35.1
N	1814	$\frac{13.1}{8}$	$\frac{33.9}{29}$	0.1	5.9	6.0
DF	729	*	$\frac{17.2}{8}$	*	*	*
D	202	$\frac{3.3}{*}$	10.4	*	*	*
F	1030	$\frac{16.4}{6}$	$\frac{40.3}{35}$	0.6	13.7	14.3
0	*	*	*	*	*	*

- (1) DFN = dogs, NO<sub>2</sub> and Fe<sub>2</sub>O<sub>3</sub> all present; DN = dogs and NO<sub>2</sub> only; FN = Fe<sub>2</sub>O<sub>3</sub> and NO<sub>2</sub> only; N = NO<sub>2</sub> only; DF = dogs and Fe<sub>2</sub>O<sub>3</sub> only; D = dogs only; F = Fe<sub>2</sub>O<sub>3</sub> only; 0 = no dogs, Fe<sub>2</sub>O<sub>3</sub> or NO<sub>2</sub> (empty chamber only).  
 (2) Based on 30-180 minute samples  
 (3) Upper figure based on 30-180 minute samples, lower on 150-180 minute samples.  
 (4) Based on 150-180 minute samples, % by weight of total sample.  
 \* Too small for analysis or significance.

Mass and molar relationships between ammonium and nitrate were examined. Graphs of  $\text{NH}_4^-$  - N and  $\text{NO}_3^-$  - N content of DFN condition samples through the 0 to 180 minute period demonstrate that the curve for  $\text{NH}_4^-$  - N closely follows the same pattern as the curve for its complementary  $\text{NO}_3^-$  - N (figure 2). In all sample sets  $\text{NO}_3^-$  - N content exceeded  $\text{NH}_4^-$  - N in average weight ratios approximating 2 (range of 1.6 - 2.3). On the basis of a 1:1 ratio of  $\text{NO}_3^-$  - N to  $\text{NH}_4^-$  - N, and of the relationships  $\text{NH}_4^-$  - N x 1.28 =  $\text{NH}_4$ ,  $\text{NO}_3^-$  - N x 4.43 =  $\text{NO}_3$ , and  $\text{NH}_4^-$  x 4.43 =  $\text{NH}_4\text{NO}_3$ , weight percentages of  $\text{NH}_4^-$  +  $\text{NO}_3^-$  assayed accountable as  $\text{NH}_4\text{NO}_3$  ranged between 45 and 65 in those conditions yielding sufficient sample weights to be meaningful (F, FN, DN, DFN). As noted above, weight percentages of total particulate accountable as the sum of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , or ammonium nitrate, ranged up to 62% with the largest percentages being in conditions of  $\text{NO}_3^-$  present with dogs and/or  $\text{Fe}_2\text{O}_3$  (DN, FN, DFN, especially DN). It stands to reason that  $\text{NH}_4^+$  +  $\text{NO}_3^-$  would account for less total particulate when the intended particulate,  $\text{Fe}_2\text{O}_3$ , is present. Why so little total particulate is accounted for by nitrogen containing ions in the other conditions, especially N, is not clear.

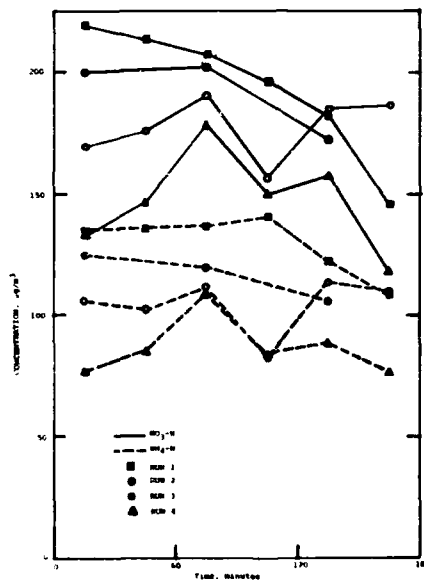


Figure 2. NITRATE AND AMMONIUM NITROGEN COMPONENTS IN CHAMBER WITH DOGS,  $\text{Fe}_2\text{O}_3$  AND  $\text{NO}_2$  (CONDITION DFN). Four sampling runs.

The observations clearly indicate the presence of particulate matter in excess of certain planned atmospheres, and suggest physical and/or chemical reactions(s) as a source. At least some of this is likely to be respirable. Under certain conditions a substantial portion of this alien material was nitrogen-bearing, and of this, ammonium nitrate,  $\text{NH}_4\text{NO}_3$ , probably assumed a major role. The nature of unaccounted-for nitrogen-containing and other total particulate was not determined but may be of sufficient interest for further study.

### Ozone Study

Total counts were obtained by the instrument for particulate size (diameter) categories:  $> 0.3\mu$ ,  $> 0.5\mu$ ,  $> 0.7\mu$ ,  $> 1\mu$ ,  $> 2\mu$ , and  $> 4\mu$ . For some of the collected data these were then corresponding derived categories:  $0.3-0.5\mu$ ,  $0.5-0.7\mu$ ,  $0.7-1\mu$ ,  $1-2\mu$ ,  $2-4\mu$ , and  $> 4\mu$ . Air was sampled from and data collected for: outdoors (designated OD; before air filtration and conditioning); in duct supplying air to chamber inlet (SD; after filtration and conditioning but before introduction of  $\text{O}_3$ ); and within the chamber under conditions of no dogs and no  $\text{O}_3$  (C),  $\text{O}_3$  but no dogs ( $\text{O}_3$ ), dogs but no  $\text{O}_3$  (D), and dogs with  $\text{O}_3$  ( $\text{DO}_3$ ). Outside and duct counts ensured that associated critical chamber counts could be compared for within-chamber effects of primary interest and were not being unduly influenced by rapid or severe fluctuations in outdoor-duct air aerosol content.

It was noted that outdoor air counts did fluctuate quite widely in time, and that this was reflected to some extent in the duct and chamber counts, specifically in the smaller size ranges ( $< 1\mu$ ). However, the proportionately small fluctuations in duct particulate count, their relatively slow temporal pattern, and frequent reference sampling along with chamber sampling, maximized the validity of chamber aerosol variations when dog- $\text{O}_3$  conditions were studied.

Chamber sampling indicated that without dogs in the chamber, the introduction of  $\text{O}_3$  in a quantity characteristically yielding approximately one ppm (0.8-1.3 ppm) in a dog-loaded chamber produced substantially greater concentrations (20-35%) than when dogs were present, and increased the total particulate counts by 18 to 73 percent. This suggests that dogs interact with and reduce the ozone introduced to their chamber atmosphere, which effect is noted commonly when  $\text{O}_3$  and other reactive agents and must be accounted for in characterizing chamber exposure conditions. It has also been observed that chamber materials (walls, inlet duct, etc.) react with  $\text{O}_3$ . Thus, it is not safe to assume that chamber animals are breathing the same concentration as sampled at the generator or chamber inlet duct. Also,  $\text{O}_3$  appears to react with chamber atmospheres even in the absence of dogs to produce alien particulate material in addition to that carried in the supply air, and in a respirable size range. Aerosol count increments of 18 to 73% were observed with  $\text{O}_3$  was introduced to chambers from which the dogs had been removed. With dogs in the chamber, introduction of the same  $\text{O}_3$  levels increased total particulate counts 11 to 81%. The total count pattern also appeared to follow that of  $\text{O}_3$  concentration when the latter was manipulated both with and without dogs. It was quite apparent that despite animal loading, introduction of gaseous  $\text{O}_3$  resulted in reactions yielding particulate matter alien to the planned atmosphere, in a manner similar to that observed in the  $\text{NO}_x$  study reported above.

That dogs alone contribute some to the chamber particulate loading was indicated by comparing ratios of chamber: duct counts and chamber counts with: without dogs, in the absence of  $O_3$ . Small to moderate increases in total particulate were observed to result from substantial surges in activity of the dogs in the chamber. This variable was accounted for, also, in relating particulate counts to other factors. In general, the dogs' presence appeared to contribute less significantly than did the introduction of  $O_3$ .

In addition to its effects in terms of total counts, i. e., all particles  $> 0.3\mu$ , introduction of  $O_3$  also was suspected of altering the size distribution of chamber particles. There appeared to be relative enhancement in the  $0.5-0.7\mu$  and possibly the  $0.5-1.0\mu$  range(s), and decreases in smaller and larger size categories, as a result of the presence of  $O_3$ . Comparisons of D and SD suspended particulate size distribution suggested that the presence of dogs increased the number of particles larger than  $0.5\mu$  diameter (especially  $> 1.0\mu$ ), and slightly decreased the number of particles in the  $0.3-0.5\mu$  category.

Frequency distributions of chamber and duct air particulates were heavily skewed to the  $0.3-0.5\mu$  extreme, with numerical median diameter (NMD) of approximately  $0.43$  and  $0.4$  respectively. These were in contrast to outside air particulates, which were distributed much more in the central size categories ( $0.5$  to  $2.0\mu$ ) and yielded an NMD of about  $0.7\mu$ . Quite obviously particle removal forces (filtration, sedimentation, impaction, etc.) in the air supply system were more efficient for the larger particles than for the smaller. More significant is the evidence that in addition to increasing total particulate loading  $O_3$  favored the enhancement of particulates in the deeply respirable size range.

#### NO<sub>2</sub>/O<sub>3</sub> Studies

While no appreciable chamber loss was observed with  $O_3$  and  $NO_2$  introduced singly, when introduced in combination  $O_3$  was reduced by 63 and 69% at expected concentrations of 1.1 and 3.2 ppm, and corresponding  $NO_2$  losses were 13 and 31% at expected concentrations of 9.9 and 14.6 ppm, respectively (Ulmer, 1967). These coincident losses were characterized by  $NO_2/O_3$  molar ratios approximating 2.0, agreeing with the theoretical chemical interaction:  $2 NO_2 + O_3 \rightarrow N_2O_5 + O_2$ . Although no aerosol studies were made in conjunction with this work, it does demonstrate production of new, additional constituents alien to the original design. That alien particulate reaction products could also result, especially in the presence of animals, might be anticipated from the foregoing  $NO_2$  and  $O_3$  observations.

## DISCUSSION

The above studies present grounds for query and conjecture in regard to inhalation toxicology and its objective of extrapolation to other subjects and circumstances, and invite further, more detailed investigation to the extent that implications may be deemed significant. How universal is the phenomenon of atmospheric reaction and formation of new, alien particulate or gaseous products? What conditions control these reactions? To what extent might these affect interpretation of biological responses and the experiment as originally conceived? That toxicity may be influenced, especially potentiated, by addition of particulate (especially submicron) or gaseous agents, and some of the mechanisms by which this occurs, is already recognized (Amdur, 1957, 1959). Of some new concern, beyond this more obvious and direct consequence, is the question concerning what effect these alien constituents may have on the use and extrapolation of effects from an experiment involving these phenomena to a situation in which these may not occur, or occur in a different way and with different results. For example, could results of a laboratory chamber toxicity study of gaseous agents, involving appreciable alien constituents, effectively be extrapolated to humans in a community, home or space vehicle in which environmental conditions (concentration, ventilation, crowding, etc.) may alter or preclude the questionable alien constituents? These are subjects for further consideration and research efforts.

## ACKNOWLEDGEMENTS

We gratefully acknowledge the technical assistance of Mssrs. Edward S. Thompson and Frederick J. Miller, DHER, CPEHS, NAPCA.

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## LIST OF SYMBOLS

Symbol or Abbreviation	Meaning
NO <sub>2</sub>	nitrogen dioxide
Fe <sub>2</sub> O <sub>3</sub>	ferric oxide (dust)
cu. ft.	cubic feet
cu. ft/min, dfm	cubic feet per minute
ppm	parts per million by volume
mg/m <sup>3</sup>	milligrams per cubic meter
NH <sub>4</sub> NO <sub>3</sub>	ammonium nitrate
m	meter
ft	lineal foot
m <sup>3</sup>	cubic meter
ft <sup>3</sup>	cubic foot
min	minute
μ	micron(s)
lpm	liters per minute
KI	potassium iodide (referring to assay method)
O <sub>3</sub>	ozone
O, D, N, F, DN, DF, FN, DFN	designations for chamber conditions, NO <sub>2</sub> study. See text.
NH <sub>4</sub> , NH <sub>4</sub> <sup>+</sup>	ammonium (ion)
NO <sub>3</sub> , NO <sub>3</sub> <sup>-</sup>	nitrate (ion)
NH <sub>4</sub> -N	ammonium nitrogen
NO <sub>3</sub> -N	nitrate nitrogen
%	percent
>	greater than
OD;SD;C, O <sub>3</sub> , D, DO <sub>2</sub>	designations for: outdoor; supply duct; chamber air and chamber conditions, O <sub>3</sub> study. See text.
μg/m <sup>3</sup>	micrograms per cubic meter
≈	approximately equal to

DISCUSSION

MR. MOBERG: Let's go on then and let's look at the third paper, "Alien Constituents in Experimental Atmospheres". I think this should have provoked quite a bit of comment, particularly in the different reactions that could occur. One would assume that some materials are completely free from any noxious causatives, and yet if you have the particulates present such as Dr. Campbell pointed out, ferric oxide, you can form other materials. This is the kind of kinetics we were talking about, so that we can see what the products are that we are looking for.

DR. LONGLEY: I am curious as to what the air exchange rate in those chambers might have been.

DR. CAMPBELL: They run about 30 per hour, once every two minutes.

DR. LONGLEY: Also, where were you measuring the concentrations of the materials in the chamber?

DR. CAMPBELL: They were measured just above the level of the dog's head. We had them on the floor part of this chamber and then we had another shelf above them, about two to three feet above them, and that was taken just above that, so they couldn't fool with our sample probes.

DR. LONGLEY: Do you think increasing the flow of air would have eliminated some of what I think I'd call artifacts in your basic experiment?

DR. CAMPBELL: Perhaps, to some extent. I might speculate that, yes, if you increase the air exchange rate you might reduce some of these what I refer to as alien constituents but what might be called superfluous agents, but also conversely, I think if you had lower exchange rates, you would see more of this phenomenon. As to the artifact of this, I did mention that the presence of the dogs themselves contributes to these atmospheres. I fully admit that, but we do see these things forming, (and we first called them "doggie nitrates"), even in the absence of animals. There was another feature about this and it rather contributes to the idea that animal metabolic products are at the root of some of this. In some of our work we have had an opportunity to observe these reactions sometime after the animals had been in the chamber and find that we get less of this phenomenon. When the animals are freshly removed, there is still some residue of these metabolic products of one kind or another and I think that the longer they are out of the chamber, the longer that you have for really complete degassing--or whatever you would like to call it--an equilibration of the chambers toward a clean situation, I think you will see less and less of these effects. But our main

concern is with the effect with the animals in there, which is the environment that we are studying.

MR. VERNOT: Did you identify the material in the  $\text{NO}_2$  exposure as ammonium nitrate?

DR. CAMPBELL: We identified part of the material as ammonium nitrate. There was more nitrate material. As I mentioned, there were no nitrites found. We did find nitrates in excess of what could be accounted for by ammonium nitrate. If we take ammonium as the beginning point and multiply it by the factors to make ammonium nitrate out of it, we still haven't accounted for all the nitrate present, and when we add up all the ammonium plus nitrate, we have not yet accounted for all of the total particulate either, so we haven't answered the whole question. There is something else there, we don't know what it is. It will be very interesting and I think that more and more ideas will pop up as more people think about it.

DR. HODGE: I have two or three questions. I wonder if we could see the first slide on which you had data, the one that gave on the lefthand side of the slide total micrograms of particulate matter per cubic meter for the various experiments that you describe as D and F and N?

(Slide)

DR. CAMPBELL: I apologize for these, they are no better than some others.

DR. HODGE: The numbers that I would like to ask about, the top line D, F, N-- that's Dog and Nitrate and Iron all present, right?

DR. CAMPBELL: Yes, sir.

DR. HODGE: Total sample as I read it says 1962 micrograms per cubic meter.

DR. CAMPBELL: That is correct.

DR. HODGE: If we come down to the 5th line of N, which I take it means "Nitrate"--  $\text{NO}_2$  in the absence of Dog, it's 1814.

DR. CAMPBELL: Correct.

DR. HODGE: Does that mean that there is almost as much particulate matter when  $\text{NO}_2$  is there as if all three of them were there?

DR. CAMPBELL: Our preliminary conclusions lead us to believe that, yes. I should mention that I think these figures should be taken fairly relatively. They are actual measurements of total particulate mass, and they are averages of several runs, so there is some credibility to them, I believe, but you might think at first that there are some inconsistencies here; why that one for instance should be so high relative to the DFN condition or vice versa, why the DFN shouldn't be any greater than the "N"

condition. I can only suggest here that the presence of dogs has a way of removing some of these agents.

DR. HODGE: Right. You look at the DN, the DF, and the D, these are the three lowest numbers in your columns.

DR. CAMPBELL: Correct.

DR. HODGE: So the dogs are picking the stuff up, aren't they--picking it out of the air?

DR. CAMPBELL: Yes, they do.

DR. HODGE: Does this mean there are more particles, or are the particles heavier?

DR. CAMPBELL: Unfortunately, these studies here do not include any work on enumeration or sizing of particles. It's only in the ozone study that I did this, and that was done without the chemistry, so I apologize for this. We were rather in a hurry and this is rather a sideline observation that interested me. I don't know, I can't say. As I mentioned for the ozone study, the dogs themselves contributed to the particulate loading, especially in the greater than one micron diameter size range. So, that is about all I can say about that. I could only speculate similar things here.

DR. HODGE: And this increase in the number of larger particles might have been a growth of particles in the chamber?

DR. CAMPBELL: This is possible. The dynamics of this sort of thing we haven't investigated either. Certainly if you begin with gases being introduced into a chamber wherein there are gaseous agents and you end up with particulate agents, something is happening fairly quickly. The point I was making is that this sort of thing is occurring in our experimental situation, but it may be quite a different thing when we consider situations such as in a community or in a home situation or space capsule or some such thing, although I don't intend to get into the space business.

MR. MOBERG: Thank you very much.

DR. LONGLEY: I would like to ask one more quick one: What about the relative humidity, were you measuring it?

DR. CAMPBELL: We were measuring and controlling this in the region of about 40 to 60 at its practical extremes. It was held quite closely to 50% R.H. It wandered at times. We had trouble with our equipment and that sort of thing, but we do try to keep it right very close to 50.

DR. LONGLEY: In all these sets of exposures?

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DR. CAMPBELL: Yes, I think we can say that it was 50 percent plus or minus ten percent. I understand your comment because this can be important in the production of particulate matter and in the nature of the particulate matter that is produced. This is well recognized and of course it just means more and more features of this thing that have to be defined.

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