

## Plant Damage and Eye Irritation from Ozone-Hydrocarbon Reactions

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Gas phase reaction products of several ozone-hydrocarbon mixtures, including monoolefins, a diolefin, and aromatics, were assessed for their relative ability to damage pinto bean plants and to irritate eyes. Plant damage was obtained from all reactions whose products, after cleavage at the double bond, contained three or more carbon atoms; ozone reactions with propylene and 2-butene produced no phytotoxicant. Injury was markedly reduced by attaching a methyl group at the double bond of a straight-chain olefin; however, there was no difference in injury between *cis*- and *trans*- forms of a given olefin. None of the reactions irritated eyes above that amount reported for clean, carbon-filtered air.

**O**XIDANT plant damage and eye irritation are two characteristics of air pollution in the Los Angeles area. Haagen-Smit *et al.* (4) first reported that plant damage indistinguishable from that found in the field could be reproduced by reaction products of ozone and certain hydrocarbons. Lachrymatory effects were experienced with some of the mixtures, but specific examples were not cited. Reviews of the effects of these products on plants and the chemistry of the reactions are discussed elsewhere (2, 5, 6).

Recent work by Arnold (7) showed that the phytotoxicants from reactions of ozone with 2-pentene and with 3-heptene were short-lived, having half lives of 3 and 15 minutes, respectively. In contrast, eye irritants produced from irradiated auto exhausts at Stanford Research Institute (3) were stable, having a half life of several hours. It was suggested (2) that the phytotoxicant and eye irritant were not the same material.

In a study of the relative phytotoxicity of several ozone-hydrocarbon mixtures as a possible aid to identifying the phytotoxicant, it became of interest to determine if eye irritants were also formed. The present paper discusses these experiments.

### Methods and Materials

**Plant Fumigation Chamber and Eye Irritation Booths.** A 16-cubic foot, glass and aluminum, fumigation chamber was constructed and operated as a stirred flow reactor as described previously (8). The chamber was set up in a glasshouse provided with activated carbon filters and air conditioning equipment. Natural sunlight was used for plant illumination and thus replaced the fluorescent tubes used in earlier experiments cited above. Maximum

illumination was about 1700 foot candles on a bright day.

Except for slight modifications, the eye irritation booths and related equipment were the same as those developed for a similar purpose by the Stanford Research Institute (3). Three such booths were constructed adjacent to the plant exposure chamber. The exhaust air from the chamber was run in an all-glass system to a manifold and then through three air flow lines, one to each eye exposure booth. The end of each line was connected to a loose-fitting, plastic face mask. A valve system near the manifold permitted switching from air in the chamber to the filtered greenhouse air, defined as "clean air," so that persons sitting in the booths could be given either the experimental fumigants or clean air. When the subjects received clean air, air from the chamber was exhausted to the out-of-doors.

The air flow rate through the chamber was 36 liters per minute and was split equally between the three booths. This rate gave a residence time in the chamber of 13 minutes.

**Fumigants.** Ozone, ozone-hydrocarbon mixtures, and acrolein, a known eye irritant, were used in these studies.

Ozone was prepared at the desired concentration by passing cylinder oxygen through a high voltage discharge tube and into the stirred flow reactor. Steady-state concentrations were measured with a Kruger photometer.

Sixteen hydrocarbons were used including mono-olefins, a diolefin, and three aromatics. These materials were selected to provide a range in numbers of carbon atoms and of configuration.

Liquid hydrocarbons were dispensed from a gear-driven hypodermic syringe (4) and carried as vapors into the stirred flow reactor in a stream of oxygen. Gaseous materials were metered into a

metered oxygen stream, and the resulting dilution was metered into the reactor. Hydrocarbon concentrations were calculated, as no instrument was available for measuring them.

Two concentrations of ozone and/or ozone-hydrocarbon mixtures were used. These were approximately 0.6 p.p.m. ozone to 10 p.p.m. hydrocarbon (low), and 2.5 p.p.m. ozone to 50 p.p.m. hydrocarbon (high).

Acrolein was diluted in water and the mixture dispensed from a syringe into a stream of oxygen as noted above. Concentrations were determined by absorbing the vapors in a buffered semicarbazide hydrochloride solution and reading the absorbance on a Beckman DU spectrometer.

**Eye Irritation Panel.** Thirty-six students, 26 male and 10 female, were selected from the student body of the Riverside campus. The only screening attempted was to use those students whose medical record showed freedom from chronic upper respiratory or eye troubles.

During exposures, the subjects wore activated carbon respirators so that they breathed clean air and only the eyes were exposed to the test mixture. Subjects were not aware of what they were receiving and were not able to see each other or view the equipment. Each student recorded the degree of irritation as none, medium, or severe on data sheets every 30 seconds during a 5-minute exposure. For statistical calculations, the values of 0, 1, and 2 were assigned, respectively, to the responses of none, medium, and severe. The maximum value recorded by a subject during a test was used as the response of that subject to a given material.

**Plant Material.** Pinto bean plants, 14 days old, were used as the test material for damage assessment. Four

**Table I. Plant Damage and Eye Irritation from Carbon-Filtered Air, Acrolein, Ozone, and Ozone-Hydrocarbon Reactions**

(13-minute residence in a stirred flow system)

Fumigant and Conc., P.P.M. <sup>a</sup>	Injury Index <sup>b</sup>		Eye Irritation, Max. = 2.000
	35 min.	70 min.	
Carbon-filtered air	0.0	0.0	0.361
	0.0	0.0	0.265
	0.0	0.0	0.088
Acrolein			
0.06 (calcd.)	...	...	0.471
1.3-1.6	...	1.0(0.8) [0.5-1.2]	1.182
2.0-2.3	...	1.1(1.0) [0.7-1.4]	1.476
Ozone	Low 1.4	3.1	0.270
	High 5.6	8.3	0.090
Propylene	Low 0.0	0.0	0.250
	High 0.0	0.0	0.235
1-Butene	Low 0.0	0.0	0.114
	High 4.1(3.9) [3.2-4.6]	5.3(5.3) [4.8-5.8]	0.152
3-Methyl-1-butene	Low 0.03(0.03) [0.02-0.04]	0.2(0.1) [0.06-0.2]	0.188
	High 7.1(7.3) [6.8-7.8]	8.6(8.7) [8.3-9.1]	0.235
<i>trans</i> -2-Butene	Low 0.0	0.0	0.303
	High 0.0	0.0	0.286
1-Pentene	Low 0.6(0.4) [0.2-0.5]	1.3(1.1) [0.8-1.4]	0.156
	High 9.2(9.5) [9.3-9.8]	9.1(9.6) [9.1-9.8]	0.382
2-Pentene	Low 0.0	0.0	0.182
	High 0.9(0.5) [0.4-0.6]	2.5(2.2) [1.7-2.7]	0.088
<i>cis</i> -3-Methyl-2-pentene	Low 0.0	0.0	0.110
	High 0.0	0.4(0.2) [0.1-0.4]	0.090
1-Hexene	Low 0.8(0.5) [0.3-0.7]	2.6(2.3) [1.7-2.9]	0.167
	High 6.7(7.2) [6.3-8.1]	9.8(9.9) [9.8-10.0]	0.281
	...	7.7(8.1) [7.5-8.6] <sup>c</sup>	None <sup>d</sup>
2-Methyl-1-hexene	Low 0.0	0.3(0.3) [0.1-0.5]	0.180
	High 0.1(0.05) [0.02-0.1]	1.1(0.9) [0.6-1.2]	0.110
	...	1.1(0.8) [0.5-1.1] <sup>c</sup>	None <sup>d</sup>
<i>cis</i> -3-Hexene	Low 0.2(0.1) [0.07-0.2]	1.3(1.1) [0.7-1.5]	0.286
	High 2.8(2.6) [2.1-3.0]	5.5(5.8) [4.7-7.0]	0.333
	...	9.7(9.8) [9.7-9.9] <sup>e</sup>	None <sup>d</sup>
<i>trans</i> -Hexene	Low 0.03(0.03) [0.02-0.05]	0.3(0.2) [0.1-0.3]	0.090
	High 1.5(1.4) [1.2-1.6]	3.4(3.4) [3.0-3.8]	0.210
	...	9.1(9.5) [9.1-9.8] <sup>e</sup>	None <sup>d</sup>
3-Heptene	Low 0.4(0.2) [0.1-0.3]	1.4(1.2) [1.0-1.5]	0.161
	High 9.8(9.9) [9.9-10.0]	9.9(9.9) [9.9-10.0]	0.212
1,3-Butadiene	Low 0.0	0.0	0.182
	High 1.0(0.8) [0.6-1.0]	1.5(1.3) [0.9-1.7]	0.353
Benzene	Low 0.5(0.3) [0.2-0.4]	1.2(1.0) [0.7-1.3]	0.167
	High 2.2(2.1) [1.8-2.3]	1.6(1.5) [2.0-2.5]	0.265
Toluene	Low 0.0	0.0	0.147
	High 0.6(0.4) [0.2-0.6]	1.3(1.1) [0.7-1.5]	0.229
Cumene	Low 0.2(0.07) [0.04-0.1]	1.0(0.7) [0.5-0.9]	0.121
	High 2.3(2.2) [2.0-2.4]	2.0(1.9) [1.7-2.2]	0.235

<sup>a</sup> Only the hydrocarbons are listed for ozone-hydrocarbon reactions. Low, 0.6 p.p.m. ozone to 10 p.p.m. hydrocarbon; high, 2.5 p.p.m. ozone to 50 p.p.m. hydrocarbon.

<sup>b</sup> Index in ( ) corresponds to average angle; index range in [ ] corresponds to 99% confidence limits for average angle.

<sup>c</sup> Average of 4 experiments.

<sup>d</sup> Regular panel not available; value is response of 4 to 6 staff members.

<sup>e</sup> Average of 2 experiments.

sets of plants were placed in the chamber during four successive 35-minute periods and two sets were exposed during two cumulative 70-minute periods. Each set consisted of five pots, each containing three plants with two primary leaves per plant, or a total of 30 leaves per set. Injury to the primary leaves of exposed plants was estimated the second day after exposure as per cent of leaf surface damaged and recorded on an injury

scale of 0 to 10, wherein 10 indicated 100% injury. For calculation of the 99% confidence limits, the scale of observations (per cent injury) was transformed to angles (angle = arcsin  $\sqrt{\text{percentage}}$ ) to stabilize the variance (7).

**Test Procedure.** Plants and eyes were exposed simultaneously to a given fumigant. The selection of fumigant and concentration was randomized and

only one combination was run on any one day. Approximately 31 subjects of the total panel were exposed each day. After a given fumigant had reached steady-state concentration, plants were placed in the chamber and the exhausts passed over the subjects' eyes. In ozone-hydrocarbon mixtures, the ozone was first brought to steady state, the hydrocarbon added, and the experiment begun when the reaction had reached steady state as indicated by the reduced ozone level on the photometer.

## Results

The results of the experiments are summarized in Table I, showing the fumigants, concentrations, plant injury indexes corresponding to the average angles, the 99% confidence limits, and eye irritation values. The injury index indicates oxidant type damage except for that produced by ozone alone, which was typical of ozone. Damage from oxidants and ozone occurs on the lower and upper leaf surfaces, respectively, and is easily distinguished on the pinto bean (5).

**Plant Damage.** Plant damage was obtained at one or both concentrations with all of the hydrocarbons except propylene and 2-butene. This is the first report of damage for the six following materials: 3-methyl-1-butene, *cis*-3-methyl-2-pentene, *cis*- and *trans*-3-hexene, toluene, and cumene. Propylene and 2-butene give reaction products of one and two carbons; plant damage has never yet been obtained in ozone reactions with such materials. Haagen-Smit *et al.* (4) proposed that the most reactive hydrocarbons, with respect to formation of phytotoxicant, were those giving reaction products with three to five carbon atoms, and even those with three were sometimes inactive. It has since been reported (2) that compounds such as 2-pentene, giving rise to three-carbon reaction products, will produce phytotoxicants if the concentration and residence time are optimal. This is further borne out by the present results with 3-methyl-1-butene and *cis*- and *trans*-3-hexene, all of which give three-carbon reaction products.

In general, there is agreement with results of previous work in that the low concentration of 1-pentene, 1-hexene, and 3-heptene approached threshold values for plant damage with approximately 1/2-hour exposures, and 1-hexene gave the most damage. At the high concentration, either at 35- or 70-minute exposures, damage from 1-pentene and 3-heptene reactions exceeded that from 3-hexene; damage from 3-methyl-1-butene and the two forms of 3-hexene also was equal to or exceeded that from 1-hexene.

Special attention was given to comparing the phytotoxicity of a straight-chain olefin with one wherein a methyl group was attached at the double bond, and cis and trans forms of a given material. For this purpose comparisons were made between 1-hexene and 2-methyl-1-hexene and between *cis*- and *trans*-3-hexene. The addition of a methyl group at the double bond markedly reduced plant damage and it is suggested that the methyl group interfered in some way with phytotoxicant formation. Changing from *cis* to *trans* forms did not alter level of phytotoxicity.

Oxidant damage was produced with about 2 p.p.m. of acrolein alone. While damage was not severe, it was definite and indistinguishable from the underside bronzing typical of oxidant damage. Haagen-Smit *et al.* (4) had shown that acrolein produced atypical damage on some of the test plants used.

**Eye Irritation.** No eye irritation was obtained with any ozone-hydrocarbon mixture that was appreciably

higher than that reported for clean air. Neither was irritation reported for ozone alone, even at high concentrations. For comparison of acrolein (a known lachrymator) with carbon-filtered air, the frequencies for none, medium, and severe irritation resembled a Poisson distribution; one of the appropriate transformations is  $y = \sqrt{x+1/2}$  (7).

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## MINERAL SUPPLEMENTS

### Copper, Nickel, and Cobalt Content of Oyster Shells

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The copper, nickel, and cobalt content in oyster shells has been found to be 33, 30, and 1 p.p.m., respectively. These quantities of copper and cobalt are too low to be significant in correcting soil and plant deficiencies by the application of oyster shells as liming material, but the cobalt present in oyster shells might be beneficial in poultry nutrition. Unlike copper, nickel is present in higher quantity in the shell of oysters than in the edible portion. The concentration of copper and cobalt from sea water to oyster shells is about the same, whereas nickel appears to be accumulated to a greater degree.

OYSTER SHELLS play a minor role in agriculture as a mineral supplement in poultry nutrition and occasionally as a soil additive in the form of lime or limestone. There does not appear to be a recorded observation on the cobalt or nickel content of oyster shells, and only a single reference to the quantity of copper in this material has been found.

A composite 10-pound sample of shells of the common oyster, *Ostrea virginica*, gathered on several occasions from different locations off the coast of Prince Edward Island, Canada, was thoroughly freed from organic matter by scrubbing with a stiff-bristle brush in fresh water. The shells were rinsed ten times in distilled water, dried overnight at 105° C., crushed, and pulverized to approximately 65 mesh. From this bulk sample a final 250-gram analytical sample was

obtained by riffing, and this was pulverized to -100 mesh. The washing and drying procedures used on these shells left such small quantities of organic matter and moisture that their presence had no effect on the concentration of metals in the parts per million range.

Standard colorimetric procedures were employed to determine nickel as nickel dimethylglyoxime in alkaline solution, and cobalt by the Nitroso-R salt procedure. After the addition of dimethylglyoxime and removal of nickel and cobalt by extraction with carbon tetrachloride, copper was determined with sodium diethyldithiocarbamate (5). The following results, in parts per million, were obtained: copper, 33; nickel, 30; and cobalt, 1.

The quantities of these metals found in sea water by different investigators show

considerable variation. This is not surprising when one considers the influence of land drainage, shore rock composition, currents, and other factors affecting the composition of sea water, as well as the difficulties in sampling and analysis for trace elements. The range of values published in recent years for copper, nickel, and cobalt in sea water is illustrated in Table I.

The figures reported by Young, Smith, and Langille (7) for copper and cobalt are of particular significance, as their samples were likewise taken from the sea around the Atlantic provinces of Canada. The average content of copper found by Young, Smith, and Langille agrees well with the mean compiled by Vinogradov (10), and their average cobalt value of 0.5  $\gamma$  per liter shows good agreement with the averages listed by