The Oxidation of Parathion to Paraoxon. II. By Use of Ozone

by F. A. GUNTHER, D. E. OTT, and M. ITTIG
Department of Entomology, University of California, Citrus Research Center and Agricultural Experiment Station, Riverside, California

A previous report (1) documented the need and progress in a search for an ideal universal oxidant and conditions to convert P=S organophosphorus insecticides to the generally biologically more active P=O compounds. This search still continues; however, the present paper describes a set of manual conditions using the oxidant, ozone[1/], which rapidly and conveniently oxidizes parathion to paraoxon. Conditions were not found to oxidize other compounds of interest to their P=O analogs with this reagent. Due to this lack of general application for several insecticides, no attempt was made to automate the oxidation reaction in conjunction with described automated anticholinesterase (anti-ChE) assay systems (3,4,5,6,7). One of these systems was used following the manual ozone oxidation procedure to determine the paraoxon product. Further confirmation in some tests came from the use of thin-layer and gas chromatography. Some tests were analyzed for the stoichiometric sulfate ion produced in the ozone reaction with parathion, with findings less than theoretical.

METHODS

Ozonizer

Ozone was produced as needed from commercial oxygen by use of a microozonizer constructed and operated after the manner of BEROZA and BIERL (8). To monitor (9) the reproducible production of ozone, the gaseous effluent from the ozonizer was bubbled into 10 ml. of 2% potassium iodide solution for measured periods and

---

1/ Suggested for this purpose by G. K. Kohn (2).

Bulletin of Environmental Contamination & Toxicology,
Vol. 5, No. 1, 1970, published by Springer-Verlag New York, Inc.

oxygen flow rates; the resulting solution was acidified with two ml. of 10% sulfuric acid and titrated with 0.1N sodium thiosulfate solution to provide a titer proportional to the amount of ozone produced per unit time. This procedure was repeated at least once a day and prior to use of the ozonizer with pesticide solutions to supply ozone production as constant as possible; if needed, ozonizer conditions were changed to bring production to the desired level. Generally, oxygen flow rate into the ozonizer was about 40 cc./minute with reaction times of from 5 to 30 minutes at 25° C.

Samples of parathion or other test pesticides for submittal to the ozonizer were solubilized as required in aqueous ethyl alcohol from about 10 to 95% alcohol.

Anti-ChE Analysis

One-ml. portions of the aqueous ethyl alcohol solutions (diluted to contain 5% or less ethyl alcohol) containing 0.005 to 0.1 μg. quantities of starting parathion were analyzed directly, after ozonization and standing at least 15 minutes to dissipate excess ozone (or blowing with nitrogen), by a simple automated system (5) for the determination of anti-ChE agents. Dilute solutions of standard paraoxon in 5% or less ethyl alcohol were used for standardization purposes in the AutoAnalyzer[2/].

GLC

Samples containing 100 to 500 μg. of parathion dissolved in 5 ml. of 10 to 95% ethyl alcohol were ozonized for 20 minutes at an oxygen flow rate of 40 cc./minute. The compounds of interest were extracted three times with 5-ml. portions of n-hexane, dried over sodium sulfate, and appropriate small aliquots were then analyzed directly by injection into a gas chromatograph equipped with a thermionic detector for phosphorus detection. Quantitation was based upon peak heights relative to those from parathion and paraoxon standards.

2/ Registered trademark, Technicon Corporation, Tarrytown, N.Y.

GLC parameters:

Instrument - Aerograph No. 1525B.

Carrier Gas - N_2 at 50 p.s.i. at the tank.

Column - 5% QF-1 on Gas Chrom Q, 70/80 mesh, 3 feet long by 1/8 inch I.D.

Conditions - Column temperature, 205° C., injector temperature, 212° C., detector temperature, 212° C., attenuation, 16.

Retention Times - Parathion, 5 minutes; paraoxon, 7 minutes.

TLC

The aliquots remaining after GLC analysis or aliquots from separately prepared samples were carefully concentrated at 50° C. with a jet of filtered dry air to 0.1 to 0.2 ml. and applied as spots to TLC plates consisting of 250-μ thick layers of fluorescent silica gel buffered to pH 7.0 (Mallinckrodt SilicAR TLC-7GF); parathion and paraoxon reference standard spots were applied also to each plate. The plates were then developed 10 cm. in a solvent mixture of n-hexane, chloroform, and methanol (7:2:1). Spots were observed and marked under ultraviolet light. Typical R_f measurements for paraoxon and parathion were 0.55 and 0.75, respectively. Uniform areas encompassing each spot on a plate were scraped off and compounds were eluted from the gel with n-hexane. Aliquots from each "spot" were then analyzed by GLC.

Sulfate Ion Determination

A method (10) using barium chloranilate as color reagent was used. For standardization, 2.0 ml. aliquots of sodium sulfate (5 to 100 μg. of sulfate ion) solution in 50% ethyl alcohol were pipetted into centrifuge tubes, followed in each by 1.0 ml. of color reagent; each tube was mixed periodically during 15 minutes, centrifuged, then read at once in a one-cm. microcell in a Beckman Model DB spectrophotometer at 530 nm. versus a 50% ethyl alcohol blank solution.

Sample solutions in 2.0 ml. of 50% ethyl alcohol following ozonization for 15 minutes at an oxygen flow rate of 40 cc./minute were analyzed similarly. Appropriate blank runs established no interference from dissolved oxygen and ozone.

RESULTS

When one-ml. dilute solutions of parathion were ozonized and analyzed directly by the automated anti-ChE analyzer, the results as tabulated in Table I indicate quantitative conversion to paraoxon. Furthermore, note that paraoxon was not destroyed by these conditions of ozonization (5 minutes at 40 cc. of O_2/minute).

TABLE I

Comparison of percent inhibition values calculated from automated anti-ChE analysis of ozonized parathion with those from paraoxon standard solutions at equivalent concentration

Conc. (μg./ml.)	Parathion + O_2 only	Parathion + O_3 [a]	Paraoxon	Paraoxon + O_3 [a]
0.005	--	24	28	30
0.006	--	29	30	--
0.008	--	36	36	--
0.010	--	41	41	42
0.025	--	64	69	--
0.050	--	80	86	88
0.1000	7	86	93	--

a/ Five minutes each with an O_2 flow rate of 40 cc./minute through the ozonizer.

Results from 5 ml. solutions of considerably higher concentrations of parathion after being ozonized for up to 30 minutes also indicated conversion of parathion to paraoxon as demonstrated by both TLC and GLC; however, calculated efficiencies for the reaction ranged from only 20 to 40% (see Table II), with higher yields for the smaller quantities.

TABLE II

Results calculated from GLC analytical data of various parathion solutions and conditions of ozonization

Ozonization time (min.) [a/]	Parathion (µg./ml.)	Ethyl alcohol (%)	Conversion of parathion to paraoxon (%)
15	20	10	30
15	100	10	20
15	20	50	35
15	100	50	28
30	20	95	40

a/ O_2 flow rate of 40 cc./minute through the ozonizer.

Similar high-concentration solutions of parathion were analyzed, after ozonization, for sulfate ion as a theoretically stoichiometric product from the reaction ($P{=}S + O_3 \rightarrow [SO_2] + O_3 \rightarrow SO_4^{--}$) and as other evidence supporting the reaction. To explore the possibility of loss of the assumed intermediate sulfur dioxide, the ozonization tube consisted of a side-arm test tube with septum closure at the top to permit puncture and introduction through it by the long needle outlet from the ozonizer; gaseous effluent from the side-arm of the reaction vessel then was scrubbed in another tube containing 50% ethyl alcohol. Contents of both tubes subsequently were analyzed separately for total sulfate ion production; sulfate ion was never found in the second tube. The results (see Table III) are reasonably consistent with recoveries of paraoxon as shown in Table II from GLC analysis.

TABLE III

Results of colorimetric determination of sulfate ion derived from parathion exposed to ozone

Sample + 1 ml. color reagent	Absorbance reading	Percent recovery of sulfate ion calculated from theoretical yield
50% EtOH	0.158	
50% EtOH + O_2 [a]	0.158	--
50% EtOH + O_3 [a]	0.156	
250 µg. parathion + 50% EtOH + O_3 [a]	0.238, 0.215 [b]	38, 34 [b]

a/ O_2 flow rate at 40 cc./minute for 15 minutes.

b/ Two separate experiments.

DISCUSSION

The results presented offer rather conclusive evidence for the production of paraoxon from parathion exposed to ozone. However, the discrepancy between the quantitative aspects of the reaction as demonstrated with low-level parathion concentrations compared to relatively high concentrations is not completely clear. Higher concentrations of parathion necessitate higher concentrations of ethyl alcohol to keep the pesticide solubilized and therefore the reaction mixtures are not exactly comparable. A similar discrepancy was noted when using silver oxide (AgO) as the oxidant (1).

Failure of ozone to effect similar reactions for other pesticides is either failure to find the proper conditions for these other compounds or that ozone is just too powerful an oxidant, resulting in destructive, competing reactions.

The exact nature of the reaction of ozone with parathion is not evident, but presumably sulfur dioxide is an intermediate. Ultimately, however, as demonstrated by the positive detection of sulfate ion, the

sulfur which was replaced from parathion by oxygen is oxidized by oxygen and/or ozone to sulfate ion.

Apparently little knowledge exists regarding the effect of ozone on pesticides. BUESCHER et al. (11) reported the destruction of aldrin, dieldrin, and lindane in aqueous solutions by ozone with no mention of end products; destruction was demonstrated by the loss of gas chromatographic response with an electron capture detector.

CONCLUSIONS

Parathion in low concentrations in aqueous solutions can be readily converted to paraoxon in high yield by use of ozone. However, the reaction is not a general one for the production of other oxons from their corresponding organothiophosphorus analogs.

ACKNOWLEDGMENTS

The authors thank R. C. Blinn for the pure paraoxon and W. E. Westlake for advice and assistance relating to the GLC aspects. Some financial assistance was generously provided by Public Health Service Research Grant CC 00315 from the National Communicable Disease Center, Atlanta, Georgia.

REFERENCES

1. F. A. GUNTHER, D. E. OTT, and F. E. HEARTH, Bull. Environ. Contamination and Toxicol. 3, 49 (1968)

2. G. K. KOHN, personal communication, Dec. 12, 1966

3. G. D. WINTER, Ann. N. Y. Acad. Sci. 87, 875 (1960)

4. D. E. OTT and F. A. GUNTHER, J. Assoc. Offic. Anal. Chemists 49, 662 (1966)

5. G. VOSS, J. Econ. Entomol. 59, 1288 (1966)

6. D. E. OTT, J. Agr. Food Chem. 16, 874 (1968)

7. D. C. LEEGWATER and H. W. VAN GEND, J. Sci. Food and Agr. 19, 513 (1968)

8. M. BEROZA and B. A. BIERL, Anal. Chem. 38, 1976 (1966); Anal. Chem. 39, 1131 (1967)

9. B. E. SALTZMAN and A. F. WARTBURG, JR., Anal. Chem. 37, 779 (1965)

10. C. M. BIRDSALL, A. C. JENKINS, and E. SPADINGER, Anal. Chem. 24, 662 (1952)

11. C. A. BUESCHER, J. H. DOUGHERTY, and R. T. SKRINDE, J. Water Pollution Control Federation 36, 1005 (1964)