

DATA EVALUATION REPORT

Photodegradation of Bronopol in Water (161-2) MRID 429413-03

CITATION: Jackson, R., Hall, B.E., Self, D.. Bronopol--Environmental Fate, Phase 4 Response, Photodegradation--Water. 2 Jul 1992. Laboratory Project No. 381834. Performed by Inveresk Research International, Ltd.; Tranent, Scotland. Sponsored by The Boots Company PLC, Nottingham, UK.

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7/11/95

CONCLUSIONS AND DATA SUMMARY (see attached for chemical structural formulas and C-14 label position)

This study is acceptable but only partially satisfies the data requirement. To be fully satisfactory it is necessary that a major degradate, component "B", be identified and its pattern of formation and decline established.

Bronopol (C-14 labeled 2-bromo-2-nitropropane-1,3-diol) at a nominal, sterile (see below) aqueous concentration of 5 ppm rapidly photodegraded at the hydrolytically stable pH 4 (citrate buffer) at 25°C under continuous artificial irradiation (nominal 450 W/m² xenon-source filtered to pass wavelengths between 290 and 800 nm) such that approximately one-half remained after about 24 hours (calculated DT₅₀ = 24.3 hr). An equivalent exposure time under natural sunlight was estimated to be approximately 2 days (assuming 12 hours each of light and dark). Parent and degradates were separated and tentatively identified by TLC and reverse phase HPLC. HPLC followed by LSC was used for definitive or "accurate" quantitation of degradation. TRIS (2-hydroxymethyl-2-nitropropane-1,3-diol, also named as tris-(hydroxymethyl)-nitromethane), a tentatively identified major degradate, increased to a maximum of about 60% of the dose after 72 hours before decreasing to about 45% by the end of the 168 hour (one week) study. Another major, but unidentified, "relatively polar" product (component "B") steadily increased, and at the end of the one week study was up to about 30% of the dose. Inspection of the data shows Component "B" was not the putative degradate 2-bromo-2-nitroethanol. Labeled carbon dioxide assumed trapped in ethanolanine monotonically increased and was up about 20% at study end. That the carbon dioxide originated from the central carbon atom of this small molecule shows that at least one reaction channel leads to extensive degradation. Although the carbon dioxide increased in parallel with unknown component "B", their formation superficially appears to occur by separate pathways. Figure 1 shows the kinetic pattern for bronopol and degradates.

There were numerous omissions and ambiguities in the reporting of this study. Description of procedures and tabulation of results were not well-organized or easy to follow. It is unlikely that the reported incidental microbial growth had any effect on results.

EXPERIMENTAL PROCEDURES

To the essentials outlined in the data summary above, the following are additional salient features:

After repurification by TLC the bronopol test material had a specific activity of 73.27 $\mu\text{Ci}/\text{mg}$ or 1.627×10^5 $\text{dpm}/\mu\text{g}$ (nuclear statistics to indicate the reliability of these activity values were not given) and a stated "radiochemical" purity of greater than 97%. Two stock test solutions were prepared in 0.01 M tri-sodium citrate buffer (pH 4). Solution concentrations determined by LSC were approximately 10 and 5 $\mu\text{g}/\text{g}$ for a preliminary and main study, respectively. Samples of approximately 20 g of solution each were used for the subsequent testing. Irradiation was through cylindrical quartz vessels in all cases.

After preliminary parameters were established, 10 vessels for irradiation and another 10 for use as dark controls were prepared for the main study. A continuous flow of carbon dioxide free air was passed through the vessels and through two ethanolamine traps to which each vessel was connected.

Duplicate vessels from the irradiated and dark controls were taken for analysis immediately after preparation (zero-time) and at 4, 12, 24, 72, and 168 hours. After transfer of test solutions and washing of the test vessels, duplicate subsamples of approximately 0.1 g, 0.5 g and 1.0 mL, respectively, of test solutions, trapping solutions, and washings were assayed by LSC.

In the preliminary study samples of test solutions were analyzed by TLC on silica gel using two solvent systems: 1) dichloromethane: methanol (9:1 by volume) and 2) toluene: propan-2-ol: acetic acid (9:1:1 by volume). Non-radiolabeled reference standards of bronopol, TRIS, and 2-bromo-2-nitroethanol were co-chromatographed with each sample and located with u.v. light. For the main study test solutions were analyzed by TLC (using only the first solvent system above), and reverse-phase HPLC. Radioactivity of liquid samples was measured by LSC. Radioactivity on TLC plates was located and quantitated by a radio-TLC analyzer.

Tests for sterility (two media) using different samples from those chemically analyzed (but prepared from the same stock solution) and incubated at 37°C were made at intervals for up to 10 days. Microbial growth appeared in 4 out of 12 irradiated samples which contained test solution and in 1 of 4 blank controls which contained growth medium only. However, since essentially no degradation was detected in the dark controls of the main study, it was surmised that the contamination was from an incidental source and did not influence results from the main study.

COMMENTS

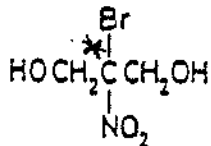
- ▶ In addition to not identifying component "B", why was the study not extended beyond one week to follow this degradate until it reached a plateau or declined?

- ▶ Inspection of the chromatographic figures provided shows poor separation of components. Resolution parameters and mathematical procedures or algorithms used to quantify peaks were not given. No estimate of the reliability (error limits) of numerical results was given and error bars were not included in plotted experimental data (see Error Treatment below).
- ▶ Tabulation of all samples actually analyzed indicating amounts or aliquots and their actual number of counts (not just dpm) used to make the numerical calculations leading to percentage distributions were not reported. Samples of all the various types of calculations using actual data which lead to final results should be provided. These data and specimen calculations should be at the reviewer's disposal for all studies.
- ▶ The indicated total recoveries (mass balances) were greater than 95% for all samples and essentially approached 100%.
- ▶ The pattern of microbial contamination reported in the procedure section above indicates that photodegradation results were not compromised.
- ▶ Is the observed degradation consistent with the absorption spectrum of bronopol?
- ▶ Was the substance trapped in ethanolamine confirmed to be carbon dioxide? Were residual bromide, nitrate, nitrite, etc. detected and measured? Would analysis have been more diagnostic if higher concentrations of bronopol had been used?
- ▶ Error Treatment. It is not unreasonable to expect that considerable effort be devoted to the assessment of the reliability of experimental results (perhaps as much as data collection?). Otherwise, the data could legitimately be regarded as worthless. In this study there was no estimation of types of error and probable accuracy of results. No error limits were given for numerical results and no error bars were indicated in plotted data. An error analysis should be given for all determined values (to include the proper use of nuclear statistics based on actual counts). Mass balance is a useful adjunct. Error limits or error bars should be given with summarized results. Conventional use of significant figures should be observed in reporting final numerical results. Since, as is often (usually?) the case, too few samples and replicated treatments are analyzed to treat in a direct conventional manner, it is desirable that a hypothetical expected error be calculated based on general experimental limitations for the various procedures, concentrations, methods, etc. For example, calculation of a maximum expected error is often a simple procedure, and would be appropriate for many studies. Only in this way can the apparent results, variations, and trends be meaningfully interpreted. Sensitivities, detection limits, selectivities and their method of determination should be clear and based on the appropriate statistics. Preliminary experiments designed for the express purpose of identifying sources of error and their limits should be considered. Calibration curves which illustrate the analytical criteria and concentration ranges are most informative. Pooling of precision data from similar samples or established methodologies taken over the course of time (even from other studies or sources) can often be used effectively.

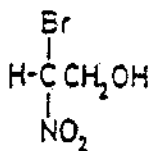
STRUCTURE OF BRONOPOL AND PUTATIVE DEGRADATES

APPENDIX 3 (continued)

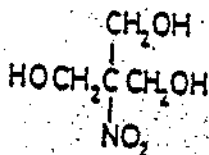
Structure of Bronopol and Putative Degradation Products



bronopol



2-bromo-2-nitroethanol



tris-(hydroxymethyl)-nitromethane

STUDY AUTHOR'S RESULTS AND DISCUSSION

Page _____ is not included in this copy.

Pages 7 through 28 are not included.

The material not included contains the following type of information:

- Identity of product inert ingredients.
- Identity of product impurities.
- Description of the product manufacturing process.
- Description of quality control procedures.
- Identity of the source of product ingredients.
- Sales or other commercial/financial information.
- A draft product label.
- The product confidential statement of formula.
- Information about a pending registration action.
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