*J. Soc. Cosmet. Chem.* 29 3–24 (1978)

# The activity and safety of the antimicrobial agent Bronopol (2-bromo-2-nitropropan-1, 3-diol)

D. M. BRYCE, B. CROSHAW, J. E. HALL, V. R. HOLLAND *and*
B. LESSEL *The Boots Company Limited, Nottingham NG2 3AA*

**Synopsis**

Recent work on the **microbiological activity, chemistry** and **safety** of the **antimicrobial** agent **Bronopol** is reported. Methods for the **estimation** of Bronopol are described, and the nature of its **decomposition** products is discussed. Animal **metabolism** and **toxicology** results are reported, together with animal and human studies on **irritancy** and **sensitisation.** The **performance** of Bronopol in a number of experimental formulations is described.

## INTRODUCTION

Early work by Hodge, Dawkins and Kropp (1) and by Zsolnai (2) suggested that geminal bromonitroalkanes had antifungal activity. The broad-spectrum antibacterial properties of 2-bromo-2-nitropropan-1,3-diol (Bronopol) have been described in a preliminary communication by Croshaw, Groves and Lessel (3) and in comparison with other members of a series of antimicrobial aliphatic halogeno-nitro compounds by Clark *et al.* (4).

Bronopol is used as a preservative in various cosmetic, toiletry and household preparations particularly because of its high activity against Gram-negative bacteria, especially *Pseudomonas aeruginosa* and other pseudomonads. These organisms are common residents in water and as such can cause contamination and spoilage problems in cosmetics and toiletries (5, 6, 7, 8). Pseudomonads are frequently implicated, particularly in oil-in-water emulsions which contain a significant amount of nonionic surfactants (9, 7, 10).

Bronopol is an effective antibacterial preservative over a wide pH range. It is stable at acid pH's and is also useful as a labile antibacterial preservative in alkaline media. Because of its broad-spectrum antibacterial activity Bronopol can also be used as an active agent, for example, in aerosol formulations. Bronopol has been reported to show persistent activity on the skin by Marples and Kligman (11), this contrasts with the fact that *in vitro* it has been shown to have a weak growth-inhibitory effect on cultured human skin cells by Onoda and Saito (12).

A programme of experimental work was begun some time ago to extend our knowledge on the safety of Bronopol. This work is now completed and it is appropriate to review the results of these and other hitherto unpublished studies on the microbiology, chemistry, analysis and formulation of this compound.

Table I. Antibacterial spectrum of Bronopol by agar-dilution in comparison with other preservative agents. Agar plates inoculated with 0·01 ml of 18 h cultures diluted 1/100 (except *Staph. aureus*); incubated for 24 h at 37°C

| Test organism | No. of species or strains | Minimal inhibitory concentration (µg/ml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Bronopol* | 6-Acetoxy-2,4-dimethyl-*m*-dioxane [1]* | cis isomer of 1-(3-chloroallyl)-3,5,7-triaza-1-azonia-adamantane chloride [2]* | Methyl Hydroxybenzoate BP† | Propyl Hydroxybenzoate BP† | Substituted imidazolidinyl urea compound [3]* | Phenylmercuric Nitrate BP† | Benzalkonium Chloride* | 8-Hydroxyquinoline sulphate† | Chlorocresol BP† | Phenoxyethanol BPC* | Formaldehyde* |
| *Gram-positive bacteria* | | | | | | | | | | | | | |
| *Staphylococcus aureus* | 1 | 12·5 | 1600 | 400 | 800 | 800 | 200 | 1·56 | 3·1 | 3·1 | 200 | > 800 | 100 |
| *Gram-negative bacteria* | | | | | | | | | | | | | |
| *Pseudomonas aeruginosa* | 2 | 12·5 | 1600 | 400 | 1600–> 1600 | > 800 | 400–800 | 12·5–25 | 800 | 400 | 400 | 800–> 800 | 100 |
| *Proteus* spp. | 4 | 12·5 | 1600 | 100–400 | 800–1600 | 200 | 200–400 | 0·78–3·1 | 25–800 | 25–100 | 200 | > 800 | 50 |
| *Escherichia coli* | 3 | 6·25–12·5 | 1600 | 100–400 | 400–800 | 800 | 200–800 | 3·1 | 25–50 | 50–100 | 100–400 | > 800 | 50–100 |
| *Salmonella* spp. | 2 | 12·5 | 1600 | 50–200 | 800 | 800 | 200 | 3·1 | 200 | 50–100 | 200 | > 800 | 50 |
| *Shigella* spp. | 2 | 12·5 | 1600 | 100–400 | 800 | 800 | 400 | 0·78 | 6·25–12·5 | 6·25–100 | 200 | 800–> 800 | 50 |

* Dilutions prepared in water.

† Dilutions prepared in 3: 1 acetone : water mixture.

[1] *Giv-Gard* DXN.

[2] *Dowicil* 200.

[3] *Germall* 115.

Table II. Antibacterial spectrum of Bronopol by agar-dilution in comparison with other antibacterial active agents. Agar plates inoculated with 0·01 ml of 18 h cultures diluted 1/100 (except *Staph. aureus*); incubated for 24 h at 37°C

| Test organism | No. of species or strains | Minimal inhibitory concentration (μg/ml) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Bronopol* | Chlorhexidine Acetate BPC* | 2,4,4′-Trichloro-2′-hydroxydiphenyl ether [1]† | 3,4′,5-Tribromo salicylanilide [2]† | N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide [3]† | Tetrabromo-*o*-cresol [4]† | Zinc salt of 2-mercapto-pyridine-1-oxide [5]† | Domiphen Bromide BP* | Cetrimide BP* | Hexachlorophane BP† | Bromchlorophene† | Dequalinium Chloride BP* | 3,4,4′-Trichlorocarb-anilide | *o*-Phenylphenol† | Chloroxylenol BPC† |
| *Gram-positive bacteria* | | | | | | | | | | | | | | | | |
| *Staphylococcus aureus* | 1 | 25 | 1·56 | 0·19 | 1·56 | 200 | 1·56 | 1·56 | 3·1 | 6·25 | 0·78 | 3·1 | 1·56 | 0·4 | 200 | 100 |
| *Gram-negative bacteria* | | | | | | | | | | | | | | | | |
| *Pseudomonas aeruginosa* | 2 | 6·25–12·5 | 25–50 | > 800 | 100–> 800 | 200–> 1600 | > 800 | 200–800 | 800 | > 800 | 12·5–50 | > 800 | 200 | > 200 | 800 | 400–800 |
| *Proteus* spp. | 4 | 12·5 | 3·1–100 | 0·39 | 25–50 | 50–400 | > 800 | 12·5–50 | 50–800 | 25–800 | 3·1–25 | 100–> 800 | 200 | > 200 | 100–200 | 50–200 |
| *Escherichia coli* | 3 | 6·25–12·5 | 3·1–12·5 | 0·39 | 50–100 | 50–200 | > 800 | 12·5–25 | 50–100 | 25–200 | 12·5–100 | > 800 | 200 | > 200 | 200–800 | 10–200 |
| *Salmonella* spp. | 2 | 12·5 | 3·1–12·5 | 0·19–0·39 | 50–100 | 100–200 | > 800 | 12·5 | 200–800 | 200–800 | 25 | > 800 | 200 | > 200 | 200 | 200 |
| *Shigella* spp. | 2 | 6·25–12·5 | 3·1–6·5 | 0·39 | 50–100 | 200–400 | 800–> 800 | 6·25 | 6·25 | 25 | 6·25–100 | > 800 | 200 | > 200 | 100 | 100 |

* Dilutions prepared in water.

† Dilutions prepared in 3:1 acetone : water mixture.

[1] *Irgasan* DP 300.

[2] *Temasept* IV (TBS).

[3] *Vancide* 89 RE (highly purified grade of captan).

[4] Deodorant K.

[5] Zinc pyrithione.

## RESULTS AND DISCUSSION

### MICROBIOLOGY

### *Antibacterial Activity*

The bacteriostatic activity of Bronopol in comparison with that of a range of other agents has been determined by serial dilution in 'Oxoid' nutrient agar (see *Tables I* and *II*). Plates were inoculated with a multi-point inoculator (13). The bacteriostatic activity against some pseudomonads, including *Ps. aeruginosa, Ps. fluorescens* and pseudomonads isolated from paints, water, cosmetics and unpreserved pharmaceutical formulations has been compared using a similar technique. Of the antibacterial agents compared only Phenylmercuric Nitrate BP and Chlorhexidine Acetate BPC had similar broad-spectrum activity to that of Bronopol (*Tables I* and *II*). 2,4,4′-Trichloro-2′-hydroxydiphenyl ether was more active than Bronopol against most of the organisms tested, but this compound was much less active against *Ps. aeruginosa.* Of the agents tested against pseudomonads only Phenylmercuric Nitrate BP was more active than Bronopol (see *Table III*).

**Table III.** Comparative activity of Bronopol and other agents against *Pseudomonas* spp. by agar-dilution. Agar plates inoculated with 0·01 ml of 18 h cultures undiluted; incubated for 48 h at 32°C

| Preservative | No. of strains tested | No. of strains with m.i.c. (µg/ml) of: | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 12·5 | 25 | 50 | 100 | 200 | 400 | >400 |
| Bronopol | 23 | 5 | 18 | 0 | 0 | 0 | 0 | 0 |
| Propyl Hydroxybenzoate BP | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 23 |
| Methyl Hydroxybenzoate BP | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 23 |
| Phenoxyethanol BPC | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 23 |
| Phenylmercuric Nitrate BP | 23 | 19 | 2 | 2 | 0 | 0 | 0 | 0 |
| Phenylethyl Alcohol BPC 1963 | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 23 |
| Benzalkonium Chloride | 23 | 0 | 0 | 0 | 0 | 0 | 19 | 4 |
| Chlorocresol BP | 23 | 0 | 0 | 0 | 0 | 0 | 23 | 0 |
| Chlorbutol BP | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 23 |
| Chlorhexidine Gluconate | 23 | 0 | 0 | 3 | 20 | 0 | 0 | 0 |
| Chlorhexidine Acetate BPC | 23 | 0 | 4 | 18 | 1 | 0 | 0 | 0 |
| 6-Acetoxy-2,4-dimethyl-*m*-dioxane [1] | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 12 |
| *cis*-isomer of 1-(3-chloroallyl)-3,5,7-triaza-1-azonia-adamantane chloride [2] | 12 | 0 | 0 | 0 | 0 | 5 | 3 | 4 |
| Substituted imidazolidinyl urea cpd. [3] | 6 | 0 | 0 | 0 | 0 | 2 | 1 | 3 |
| N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide [4] | 12 | 0 | 0 | 0 | 0 | 3 | 2 | 7 |
| Zinc salt of 2-mercaptopyridine-1-oxide [5] | 12 | 4 | 1 | 2 | 0 | 2 | 2 | 1 |
| 2,4,4′-Trichloro-2′-hydroxy-diphenyl ether [6] | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |

[1] *Giv-Gard* DXN.
[2] *Dowicil* 200.
[3] *Germall* 115.
[4] *Vancide* 89 RE.
[5] Zinc pyrithione.
[6] *Irgasan* DP 300.

The effects of organic matter and some possible antagonists are shown in *Table IV*. Bryce and Smart (14) reported that nonionic surface active agents, e.g. polysorbate 80 and lecithin, have little or no effect on the antibacterial activity of Bronopol, although such agents are known to antagonise the action of many preservatives and Brown (15) confirmed that a plot of activity *versus* the log phase concentrations of *Ps. aeruginosa* for solutions of Bronopol containing 1% polysorbate 80 showed that activity did not decrease. Sulphydryl compounds are markedly antagonistic to the *in vitro* activity of Bronopol (3). This has been confirmed by Stretton and Manson (16).

**Table IV.** The effect of organic matter and possible antagonists on the bacteriostatic activity of Bronopol by Agar dilution (strains of *Pseudomonas aeruginosa* test organism)

| Additive | Decrease (-fold) in bacteriostatic activity* |
|---|---|
| 10% ox serum | 0–2 |
| 50% ox serum | 4–8 |
| 10% human serum | 2 |
| 50% human serum | 4 |
| 10% oxalated horse blood | 4–8 |
| 50% oxalated horse blood | 32–64 |
| 10% milk | 0 |
| 1% polysorbate 80 | 0 |
| 0·1% lecithin | 0 |
| 0·1% cysteine hydrochloride | 16–64 |
| 0·1% sodium thioglycollate | 8–16 |
| 0·1% sodium thiosulphate | 4–16 |
| 0·01% sodium metabisulphite | 8–16 |

* 2-fold serial dilution.

Using the filter paper strip technique (17) with *Staphylococcus aureus* as the test organism, it has been shown that there was no inhibition of the activity of Bronopol by Cetrimide BP, Domiphen Bromide BP, Benzalkonium Chloride BPC or trichlorocarbanilide.

Further work has confirmed the report by Croshaw *et al.* (3) that there is no evidence of the development of Bronopol-resistant organisms after passage in the presence of Bronopol for 20 subcultures. In practice Bronopol-resistant organisms have not occurred.

Some insight into the mode of action of Bronopol has been obtained. Since Bronopol is more active against metabolising cells than resting cells and its antibacterial activity is reversed by thiol-containing compounds (3), thiol-containing enzymes would appear to be implicated. Bronopol forms disulphide bonds from thiol groups and these may account for the observed inhibition of dehydrogenase activity by the compound at concentrations approximating the minimum inhibitory value for each organism. Inhibition of microbial membrane-bound dehydrogenase enzymes may cause alterations in membrane structure and account for the cell leakage observed on Bronopol treatment (16). Thus thiol-containing enzymes are involved in the mode of action of Bronopol against bacteria. The selectivity of the compound for micro-organisms, indicated by its very low mammalian toxicity, may be due in part to the rapid metabolism of Bronopol by the body tissues.

## CHEMICAL AND ANALYTICAL

### *Stability of Pure Bronopol*

Experiments were conducted to establish the stability of Bronopol on storage in the pure solid state. The results, shown in *Tables V* and *VI*, demonstrate no evidence of instability over a period of one year's storage at temperatures up to 45°, and at elevated humidity. No photodecomposition was observed at room temperature over this period. Samples of Bronopol stored in the dark at room temperature over periods up to 2 years also show no evidence of decomposition. All the assays were carried out by gas-liquid chromatography (g.l.c.) using the trimethylsilylation procedure described later in the section on analytical methods. Both the internal standard and normalisation methods were utilised. Initial assays were by g.l.c. using n-pentadecane as the internal standard after acetylation. Since that time, the trimethylsilylation procedure described later has been developed in order to achieve an improvement in precision. Assays after storage were done by this latter method, using the internal standard technique.

**Table V.** Stability of Bronopol during storage

| | g.l.c. (internal standard method) | g.l.c. (normalisation method) | |
|---|---|---|---|
| Storage conditions | Bronopol % | Bronopol % | Impurity at $R_T$(rel) 0·62 % |
| Initial | 100·0 | 99·6 | 0·45 |
| 4 weeks, | | | |
| at 20 to 25°C | | | |
| (a) at normal RH | 99·5 | 99·6 | 0·43 |
| (b) at 90% RH | 100·3 | 99·6 | 0·41 |
| at 37°C | 99·9 | 99·6 | 0·39 |
| at 45°C | 99·7 | 99·6 | 0·39 |
| in north window | 100·4 | 99·6 | 0·43 |
| 8 weeks | | | |
| at 20 to 25°C | | | |
| (a) at normal RH | 99·7 | 99·6 | 0·40 |
| (b) at 90% RH | 99·9 | 99·6 | 0·39 |
| at 37°C | 100·0 | 99·6 | 0·41 |
| at 45°C | 99·4 | 99·6 | 0·41 |
| in north window | 100·1 | 99·6 | 0·44 |
| 12 weeks | | | |
| at 20 to 25°C | | | |
| (a) at normal RH | 99·8 | 99·5 | 0·49 |
| (b) at 90% RH | 100·1 | 99·6 | 0·41 |
| at 37°C | 100·2 | 99·5 | 0·46 |
| at 45°C | 99·6 | 99·6 | 0·44 |
| in north window | 99·8 | 99·6 | 0·44 |
| 52 weeks | | | |
| at 20 to 25°C | | | |
| (a) at normal RH | 100·2 | 99·5 | 0·50 |
| (b) at 90% RH | 99·2 | 99·5 | 0·47 |
| at 37°C | 100·3 | 99·5 | 0·46 |
| at 45°C | 100·3 | 99·5 | 0·46 |
| in north window | 99·3 | 99·4 | 0·51 |

RH = relative humidity.
$R_T$(rel) = relative retention time.

**Table VI.** Stability of Bronopol after storage at room temperature in the dark

| Initial assay Bronopol % | Time of storage | Assay after storage, Bronopol % |
|---|---|---|
| 99·2 | 24 months | 98·2<br>99·2 |
| 101·1 | 22 months | 101·5<br>102·2 |
| 100·2 | 21 months | 101·4<br>102·4 |
| 99·9 | 18 months | 99·7<br>99·5 |

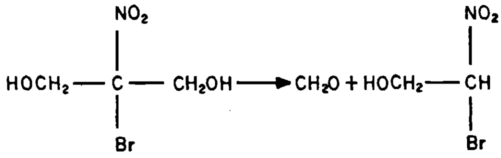
### *Stability in Aqueous Solution*

Bryce and Smart (14) have shown that aqueous solutions of Bronopol are reasonably stable when acid. To investigate the stability of the compound in more detail, aqueous solutions of Bronopol (0·2% w/v) were prepared at pH 4 and 6 (McIlvaine buffer) and pH 8 (phosphate buffer). The solution at pH 4 was stored in the dark at 50°C, the solution at pH 8 at 30°C, and the solution at pH 6 at 30, 40 and 50°C. At appropriate time intervals aliquots were removed and examined microbiologically, polarographically, gas-chromatographically and for bromide ion, nitrate ion, nitrite ion and formaldehyde. Aqueous solutions of Bronopol (10% w/v) at about pH 6 were stored at temperatures ranging from 40 to 100°C, the pH being maintained by the addition of 5N sodium hydroxide. Aliquots were removed and examined by thin-layer chromatography (t.l.c.) and bioautography. Attempts were made to isolate the decomposition products from partially decomposed solutions by preparative layer and Sephadex column chromatography.

The decomposition of Bronopol was found to be accelerated by increasing the pH or the temperature of the solution. These effects are shown graphically in *Figs. 1* and *2*; using a factor of approximately 4 as the increase or decrease in the rate of decomposition per 10°C temperature change, the times for 50% decomposition extrapolated to 20°C, and based on the g.l.c. assay results, are as shown below:

| pH | Time for 50% decomposition |
|---|---|
| 4 | >5 years |
| 6 | 1½ years |
| 8 | 2 months |

The initial process in the decomposition of Bronopol appears to be a retroaldol reaction with the liberation of formaldehyde and the formation of bromonitroethanol:



$$HOCH_2-\overset{NO_2}{\underset{Br}{C}}-CH_2OH \longrightarrow CH_2O + HOCH_2-\overset{NO_2}{\underset{Br}{C}}H$$

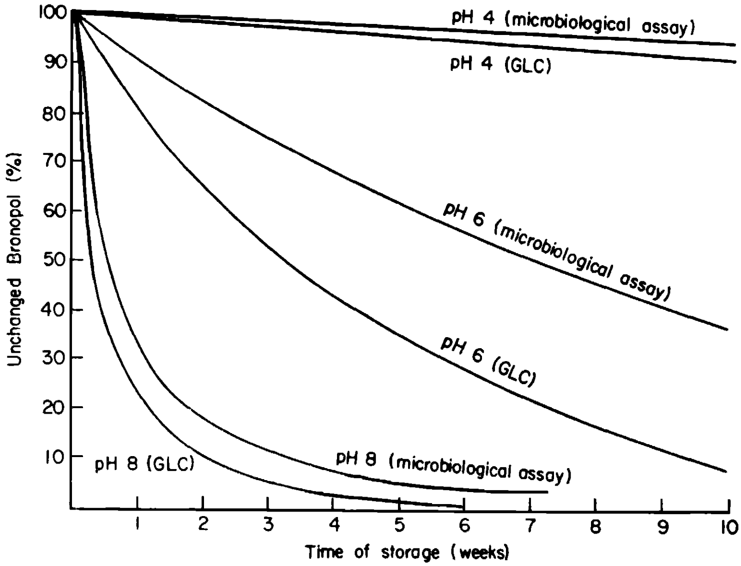


Figure 1. Effect of pH on stability of aqueous solutions of Bronopol at 40°C (initially 0·2% w/v)

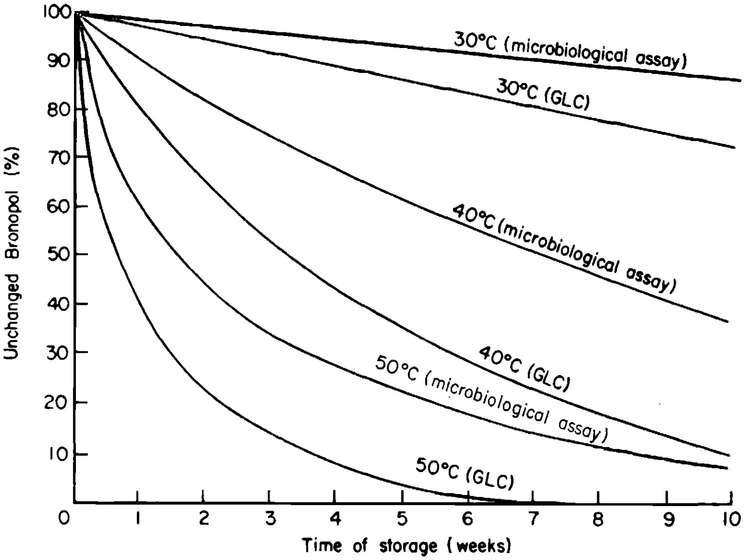
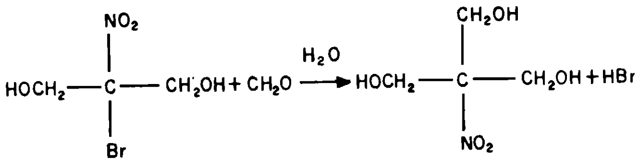


Figure 2. Effect of temperature on stability of aqueous solutions of Bronopol at pH 6 (initially 0·2% w/v)

Bromonitroethanol itself is considerably less stable than Bronopol and in the range of conditions investigated the maximal concentrations did not exceed 0·5% of the initial Bronopol concentrations.

Simultaneously a second-order reaction involving Bronopol and formaldehyde occurs to give 2-hydroxymethyl-2-nitro-1,3-propanediol:



$$HOCH_2-\overset{NO_2}{\underset{Br}{C}}-CH_2OH + CH_2O \xrightarrow{H_2O} HOCH_2-\overset{CH_2OH}{\underset{NO_2}{C}}-CH_2OH + HBr$$

2-hydroxymethyl-2-nitro-1,3-propanediol has been isolated from partially decomposed 10% w/v solutions of Bronopol by preparative layer and Sephadex column chromatography. The n.m.r. and i.r. spectra and the elemental analysis support the proposed structure. 2-Hydroxymethyl-2-nitro-1,3-propanediol itself decomposes with the loss of formaldehyde. This reaction is relatively slow, however, so that after 2–3 Bronopol half lives this compound accounts for 8–10% of the organic material, as shown by the thin layer chromatogram (*Fig. 3*).

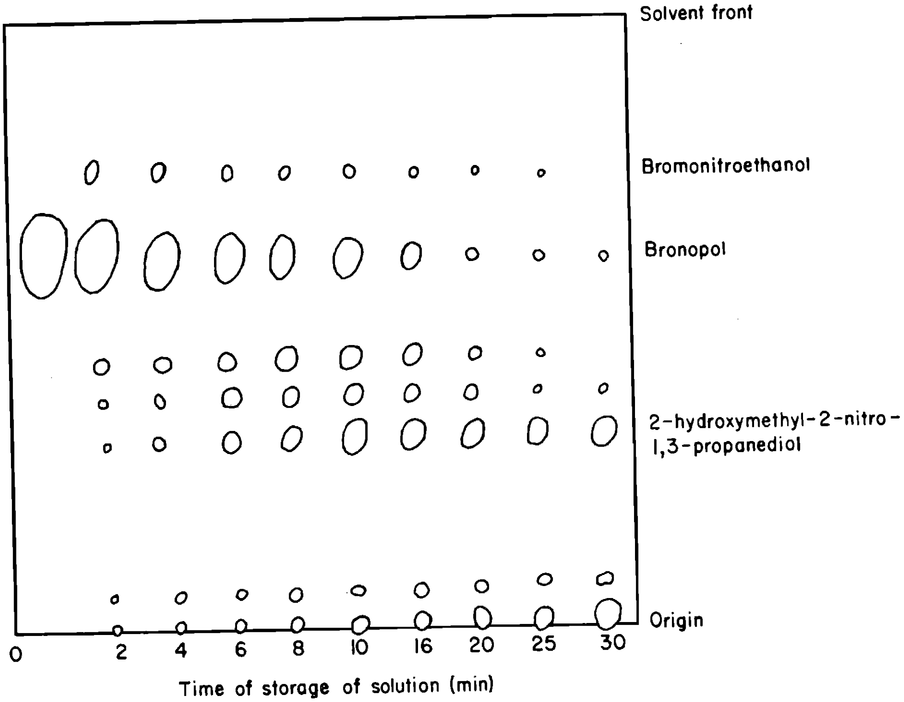


**Figure 3.** Thin-layer chromatogram of Bronopol aqueous solutions (initially 10% w/v) stored at 100° and maintained at pH 6

In more dilute solutions the second order reaction will be less important and the loss of bromine follows first order kinetics, the rate of loss being about one-half of the overall rate of decomposition of Bronopol.

A number of reactions involving formaldehyde occur simultaneously. The overall result is that the formaldehyde concentration tends to a maximum which is lower than an equimolar ratio. The rate of formation of formaldehyde relative to the rate of decomposition of Bronopol was not markedly affected by pH over the range investigated.

An additional mode of decomposition results in the formation of nitrite but not nitrate. The rate of formation of nitrite tends to follow second-order kinetics and is slower than the overall decomposition of Bronopol as measured by g.l.c. No information has been obtained on the route by which the nitro group is lost and the final organic products have not been identified. Their physical properties suggest, however, that some may be polymeric. It should be noted that in the presence of certain secondary and tertiary amines and amides, nitrite can form nitrosamines which may be carcinogenic. In the opinion of the authors, it is advisable that formulators using Bronopol, or any other substance giving rise to nitrite, take steps to ensure that if nitrosamines are produced, their presence does not represent a health hazard to the user.

The gaseous decomposition products of Bronopol have been examined by mass spectroscopy. Only three major peaks were found the first of which could be attributed to nitrogen plus a trace of ethylene at m/e 28 and the second to nitric oxide at m/e 30. The only constituent of the third peak, at m/e 44, which could be identified was the radical $CH_2NO$. Neither carbon monoxide nor carbon dioxide could be detected.

Thin-layer chromatograms of stored solutions of Bronopol were sprayed with starch/potassium iodide solution, which would locate components including those containing the aliphatic nitro group. At least seven components were detected (*Fig. 3*), three of which were identified. The origin contained sodium bromide and sodium nitrite. Bioautography, on the other hand, showed only two active zones corresponding to Bronopol and bromonitroethanol (*Fig. 4*).

It is difficult to explain the g.l.c. and microbiological results on the basis of the above observations since the bromonitroethanol and formaldehyde present are not sufficient to account for the difference between them.

### *Analytical Methods*

The methods described in this section have been used to obtain the results recorded in the preceding sections, and to assay Bronopol in the types of formulations in which it is likely to be incorporated.

Pure Bronopol has been assayed by the determination of its bromine content, by the determination of its nitrogen content and by g.l.c. of the acetylated and of the trimethylsilylated material, the methods using g.l.c. being the most specific. In formulations, Bronopol has been estimated by t.l.c., by a polarographic procedure, by a microbiological procedure and by g.l.c. The procedure by t.l.c. has been applied to ointments (at a concentration of 0·1%), to barrier creams (at concentrations of 0·1 and 0·2%) and to aerosol concentrates (at a concentration of 0·05%). G.l.c. has been applied to aqueous formulations (at concentrations of from 5 to 50 ppm). The polarographic procedure has been applied to ointments, suppositories, creams and gels (all at a concentration of 0·2%) and has also been used to estimate Bronopol in buffered aqueous solutions and in blood serum. The microbiological procedure has been applied to creams, including barrier creams (at concentrations of 0·1 and 0·2%), to liquid shampoos and also to buffered aqueous solutions. The polarographic method estimates the alkyl nitro group and therefore, although an acceptable procedure for freshly-prepared formulations, is not

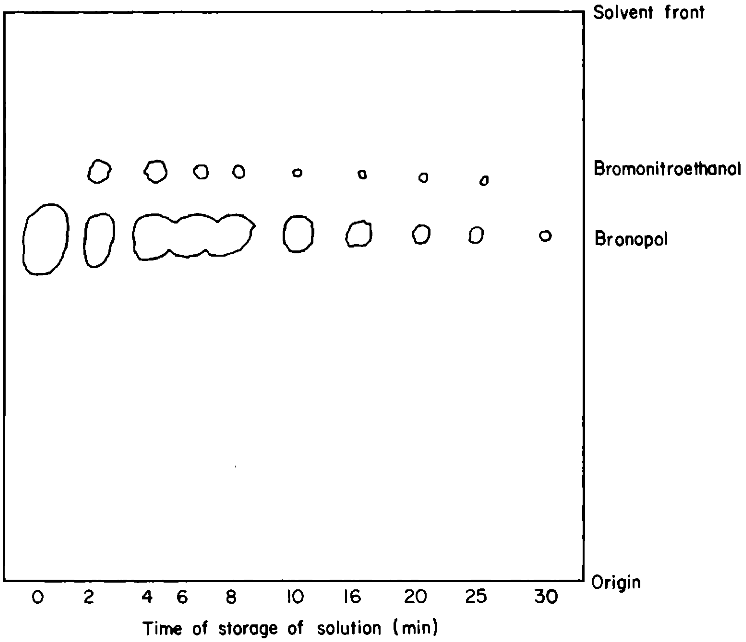


**Figure 4.** Autobiogram of Bronopol aqueous solutions (initially 10% w/v) stored at 100° and maintained at pH 6 (test-organism: *Pseudomonas aeruginosa*)

very specific and is subject to interference from breakdown products. In the absence of interfering substances, the precision of this procedure is about ±2%. The method using t.l.c. is more specific, but the spot-comparison procedure that has been used is liable to relative errors of about 15%; errors of this magnitude may be acceptable, however, at the concentrations in which Bronopol is usually incorporated in formulations. The microbiological method had an error of about ±10% on aqueous solutions and about ±10 to 20% on creams.

*Polarographic Assay.* The base electrolyte was McIlvaine's buffer solution containing 2% v/v of 0·2% v/v Triton X 200 as a maximum-suppressor. McIlvaine's buffer solution, pH 4, was prepared by mixing 12·29 parts by volume of 0·1M citric acid solution and 7·71 parts by volume of 0·2% disodium phosphate ($Na_2HPO_4$) solution. The test solution was prepared as follows. Aqueous solutions only required dilution with base electrolyte to a Bronopol concentration between $10^{-3}$ and $10^{-5}$M. Bronopol in gels, creams and other fatty-base formulations could be extracted with base electrolyte by warming gently on a steam-bath, after which any insoluble matter in the aqueous phase was removed by centrifuging and the aqueous phase diluted to give appropriate concentrations of Bronopol. Other formulations were more appropriately treated by dissolving in chloroform and extracting with base electrolyte.

The determination was carried out by transferring a portion of the solution in base electrolyte to the cell of a suitable polarograph. A stream of oxygen-free nitrogen was passed through the solution for 10–15 min to remove dissolved oxygen. The height of the mercury reservoir was adjusted to give a constant drop rate appropriate to the apparatus, this drop rate being identical with that used for the preparation of the calibration curve.

The polarogram was recorded over the range 0 to −1 V relative to the quiescent mercury pool using the appropriate recorder or galvanometer sensitivity to give a suitable wave. The diffusion current at −0·8 V relative to the mercury pool was measured, and the concentration of Bronopol read from the calibration curve.

Since the polarographic response was affected by the composition of the test solution, it was necessary to prepare a calibration curve for each formulation examined. Such calibration curves were obtained by adding known amounts of Bronopol to blank formulations and processing in the required manner.

The following are examples of the assay method which have been used.

*Microbioloical Assay.* Bronopol can be assayed microbiologically by agar diffusion using *Ps. aeruginosa* in agar of the following composition:

| | % w/v |
|---|---|
| Dextrose | 0·1 |
| Lemco beef extract | 0·15 |
| Difco yeast extract | 0·3 |
| Sodium chloride | 0·5 |
| Difco casitone | 0·08 |
| Magnesium sulphate ($7H_2O$) | 0·004 |
| Oxoid peptone | 0·6 |
| Davis agar | 1·8 |
| Distilled water | to 100, pH adjusted to 5·3. |

Alternatively Difco Assay Agar No. 11 (pH 7·9) with *Bacillus subtilis* NCIB 8054 can be used. The minimum detectable level of Bronopol in water with *Bacillus subtilis* is 0·005%. A rapid diffusion method using *Bacillus stearothermophilus* has been described by Kabay (18).

*Gas-liquid Chromatographic Assay.* Although Bronopol is a water-soluble compound it can be extracted from aqueous solution into diethyl ether or ethyl acetate after the addition of sodium chloride. The extract can then be evaporated to dryness, the residue acetylated and the Bronopol estimated by means of g.l.c. with electron-capture detection. This procedure offers a means of determining Bronopol in aqueous formulations and has been applied to Bronopol concentrations down to 5 ppm.

In aqueous formulations containing concentrations of Bronopol down to 50 ppm, the Bronopol has been determined by a similar procedure, but using n-pentadecane as the internal standard, acetyl chloride in chloroform as an acetylating reagent, carbon disulphide as the final solvent and flame ionization detection.

The following are examples of the methods which have been used to assay Bronopol by g.l.c.

*Based on the acetylated material.* The sample (about 0·15 g accurately weighed) was dissolved in 15 ml of chloroform with the aid of minimum heating, 5 ml of a 2% solution of n-pentadecane (as internal standard) in chloroform was added and the solution diluted to 25 ml. To 1 ml of this solution in a vial was added 0·3 ml acetyl chloride and the vial was sealed and then heated on a steam bath for 3 h. The mixture (2 µl) was subjected to g.l.c. in a glass column (183 cm × 3 mm) packed with 10% of silicone JXR on Gas Chrom Z (70 to 80 mesh), operated at 150°C with nitrogen (20 ml $min^{-1}$) as carrier gas and flame ionisation detection. The ratio of the product of the peak height and retention time for the Bronopol diacetate (relative retention time = 1·00) to that for n-pentadecane (relative retention time = 1·54) was calculated and compared with the ratio for a standard containing purified Bronopol which had been similarly treated. The relative standard deviation of the method was found to be 1·5%.

*Based on the trimethylsilylated material.* The sample (about 0·15 g, accurately weighed was dissolved in 15 ml of chloroform with the aid of minimum heating, 4 ml of a 1·4% solution of n-tridecane (as internal standard) in chloroform was added and the solution diluted to 25 ml. To 1 ml of this solution in a vial was added 0·1 ml of silylating reagent (prepared by mixing trifluoroacetic acid (1 part) and hexamethyldisilazane (2 parts) and filtering the mixture rapidly under dry conditions), the vial was sealed and then heated on a steam bath for 1 h. The mixture (1 µl) was subjected to g.l.c. in a glass column (152 cm × 3 mm) packed with 10% of silicone JXR on Gas Chrom Q (80 to 100 mesh), operated at 125° C. with nitrogen (40 ml $min^{-1}$) as carrier gas and flame ionisation detection. The ratio of the product of the peak height and retention time for Bronopol di(trimethylsilyl)ether (relative retention time = 1·92) to that for n-tridecane (relative retention time = 1·00) was calculated and compared with the ratio for a standard containing purified Bronopol which had been similarly treated.

*Thin Layer Chromatographic Assay.* 10% Bronopol solution was examined by t.l.c. and bioautography using 0·25 mm Kieselgel 'G', with chloroform/methanol (4 : 1) as developing solvent. 2 µl aliquots of the solution were spotted on the plates. A similar method for ointment formulations has been devised using an initial water:chloroform extraction system to remove excipients, followed by chromatography on Kieselgel $GF_{254}$ using isopropanol as the developing solvent.

*Determination of Bromide ion.* Bromide ion was determined by potentiometric titration. Bronopol solution (5 ml) was acidified and titrated with 0·02M silver nitrate solution.

*Determination of Formaldehyde.* Formaldehyde was determined by reaction with chromotropic acid. 0·2% Bronopol solution (0·5 ml) was diluted to 25 ml with 12N sulphuric acid. To this solution (1 ml) was added a 5% solution of chromotropic acid in 12N sulphuric acid (1 ml) and the mixture heated at 100°C for 30 min. Concentrated sulphuric acid (2 ml) was added and the absorbance at 570 nm measured against the appropriate blank.

*Determination of Nitrite and Nitrate.* Nitrite and nitrate were determined by reaction with 2,6-xylenol before and after decomposition of nitrite with sulphamic acid. This method was not used after the preliminary work as the results were in good agreement with the polarographic estimation of alkyl nitro-groups.

## TOXICOLOGY

### *Metabolism*

After oral administration of [$^{14}$C]Bronopol, radioactivity was rapidly absorbed and evenly distributed in tissues of the rat and dog, Moore *et al.* (19). Excretion was also rapid, the majority of the dose being excreted within 24 h.

Bronopol was rapidly and extensively metabolised so that no unchanged compound was detected in plasma and urine. It has been shown *in vitro* that Bronopol is unstable in plasma. The major urinary metabolite, accounting for more than 40% of administered radioactivity, was 2-nitropropane-1,3-diol. Other minor metabolites have not been identified. Complete metabolism was demonstrated by the finding of significant amounts of radioactivity in expired air and the appearance of a small amount of radioactivity in the tissues of dosed animals.

When applied in acetone solution to rat skin, a smaller proportion of the dose was absorbed than when dosed orally, Moore *et al.* (20). This may in part be due to the small area of skin to which the dose was applied.

When [$^{14}$C]Bronopol was applied in acetone solution to the skin of rabbits, the radioactivity was mainly localised on the epidermis around hair follicles, suggesting that limited percutaneous absorption may occur through the hair follicles.

The pattern of urinary metabolites was similar when the compound was administered orally or percutaneously, indicating no difference in metabolism related to the route of administration.

### *Acute Toxicity*

Bronopol administered in single doses by the oral and intraperitoneal routes to rodents caused gastrointestinal lesions and peritonitis. The LD50 values are shown in *Table VII.* A small number of rats were injected subcutaneously with Bronopol and those that died had haemorrhage and oedema at the site of injection, stomach lesions and lung congestion and oedema. The LD50 was approximately 200 mg/kg. After dermal application to rats of acetone solutions of Bronopol using the procedure of Noakes and Sanderson (21), death occurred at 160 mg/kg or more.

Oral administration of single doses of 40 or 100 mg/kg to dogs caused gastric irritation but no permanent injury.

No methaemoglobinaemia was observed in cats over a 24 h period following a maximum single oral dose of 25 mg/kg of Bronopol, whereas a marked rise in blood methaemoglobin concentration followed 20 mg/kg of acetanilide.

### *Chronic Toxicity*

In repeated-dose studies the observations and laboratory investigations generally included signs of poisoning, body-weight, food consumption, haematology, blood biochemistry, ophthalmoscopy, organ weights, macroscopic appearance at autopsy and histopathology, Gastrointestinal lesions, respiratory distress and some deaths resulted from daily administration of 80 or 160 mg/kg of Bronopol by oral intubation to male and female rats for 90 days whereas doses of 20 mg/kg were well tolerated. When Bronopol was given in the drinking water, rats maintained on 160 mg/kg/day for six weeks had a reduced water intake and slightly enlarged kidneys while among those given the highest dose level of 300 mg/kg/day a few deaths occurred. In dogs given a maximum daily dose of 20 mg/kg by oral intubation for 90 days, apart from some vomiting, there were no significant toxic reactions.

Aqueous 2·5% methyl cellulose solutions containing 0·2 or 0·5% Bronopol were applied once daily at a dosage of 1 ml/kg for 3 weeks to the clipped and abraded dorsal skin of rabbits. The vehicle alone and the 0·2% Bronopol solution elicited local skin erythema and the 0·5% Bronopol solution produced moderate erythema, oedema and scabbing, otherwise the rabbits showed no ill-effects clearly attributable to treatment.

**Table VII.** Acute toxicity of Bronopol to mice and rats

| Species | Sex | Route | LD50 mg/kg |
|---|---|---|---|
| Mouse | male | oral | 374 |
| | female | oral | 327 |
| Mouse | male | i.p. | 34·7 |
| | female | i.p. | 32·8 |
| Rat | male | oral | 307 |
| | female | oral | 342 |
| Rat | male | i.p. | 22·0 |
| | female | i.p. | 30·2 |

i.p. =intraperitoneal.

### *Carcinogenicity*

A carcinogenicity study was carried out in mice by application of 0·3 ml of aqueous-acetone solutions containing 0·2 or 0·5% Bronopol to the shaved backs three times weekly for 80 weeks. The concentrations were selected after a preliminary tolerance study showed that 1% or more evoked a local skin reaction. Bronopol did not alter the spontaneous tumour profile either locally or systemically.

A 2-year toxicity and tumorogenicity test, in which rats received 10, 40 or 160 mg/kg daily in the drinking water, provided no evidence to suggest that the administration of Bronopol affected tumour incidence. There was no indication of toxicity at 10 mg/kg/day, whereas the higher dose levels adversely affected growth, food intake and survival rate. Renal changes associated with diminished water intake, histological reactions in stomach and gastric lymph nodes probably due to irritancy from prolonged exposure to Bronopol, and an exacerbation of spontaneous morphological alterations in the salivary gland were also observed at the higher dose levels in a dose-related manner.

### *Reproduction Studies*

The effect of Bronopol on reproduction was investigated in rats and rabbits. In rats dosed from day 1 to 20 of pregnancy with 10, 30 or 100 mg/kg daily by oral intubation, no embryotoxic or teratogenic effects were seen even though the dams had a dose-related retardation in bodyweight gain and some died from gastric and lung lesions. A slight delay in calcification of the foetal skeleton was observed at the highest dose level. Daily application to the clipped dorsal skin of rats of 0·5 or 2% aqueous solutions of Bronopol thickened with 2·5% methylcellulose in a dose of 1 ml/kg from day 6 to 15 of pregnancy had no adverse effects on the dams or foetuses apart from causing local skin reactions at the site of application.

Oral administration of 1, 3·3 or 10 mg/kg daily to rabbits from day 8 to 16 of pregnancy also failed to produce embryotoxic or teratogenic effects though the highest dose level suppressed weight gain by the does during the dosing period.

Bronopol, 20 or 40 mg/kg daily, given orally to rats from day 15 of gestation and throughout lactation did not affect parturition, litter size or postnatal survival and development of the young. Fertility and general reproductive performance of rats were unimpaired by these dose levels given to males from 63 days before mating and females from 14 days before mating up to day 12 of pregnancy or until the litters were weaned

21 days *post partum*. In this study bodyweight gain of the males that received 40 mg/kg daily was slightly reduced.

### *Mutagenicity*

Bronopol did not exhibit mutagenic activity under *in vitro* or *in vivo* conditions. It was tested using *Salmonella typhimurium* in the 'Ames' system and in the host-mediated assay in mice; in a dominant lethal assay in mice, the only noteworthy finding was antifertility arising from toxicity rather than dominant lethality.

### *Irritancy and Contact Sensitivity*

*Animal Studies.* Preliminary studies on irritancy and contact sensitisation have been reported by Croshaw *et al.* (3).

Bronopol was tested for local effects to the mucous membrane of the eye in rabbits. A concentration of 0·5% in normal saline was non-irritant when applied once daily for four successive days, whereas solutions in polyethylene glycol 400 were irritant at 5% but not at 2% following a single application.

Skin irritancy was investigated by application of Bronopol in a variety of solvents to the non-abraded, clipped and shaved backs of rabbits for 6 h, with or without occlusion. Acetone solutions of Bronopol were non-irritant at 1% when given as a single application under occlusion though highly irritant at 0·5% on repeated application without occlusion. Similar results were obtained with Bronopol in 2·5% aqueous methylcellulose solution tested under these conditions at 0·5% concentrations. Bronopol in polyethylene glycol 300 was non-irritant at 5% as a single application with occlusion. These findings indicate that the irritancy of Bronopol to the skin is dependent upon the vehicle employed, thus it would be advisable to test each new formulation containing Bronopol for local effects on topical application.

Bronopol was without skin-sensitising activity in the guinea pig when tested as a 1% solution in acetone by the ear-flank method (Stevens (22)), whereas dinitrochlorobenzene was strongly positive.

*Human Studies.* The skin irritant effect of Bronopol was investigated both on volunteers and on patients attending a contact dermatitis clinic.

The volunteer study showed that Bronopol is slightly irritant to human skin at 1% in soft paraffin (petrolatum), and at 0·25% in aqueous buffer at pH 5·5. The study consisted of a closed patch test using 1 cm lint squares backed with Blenderm surgical tape on the forearms of ten subjects. Concentrations of 0, 0·5, 1 and 2% Bronopol in soft paraffin and of 0, 0·05, 0·1 and 0·25% in aqueous buffer at pH 5·5 were used. Any skin reaction after 24 h was graded from 0 (=normal skin) to 5 (=marked erythema with vesicles and induration). The results are shown in *Table VIII*. The study carried out on patients attending a contact dermatitis clinic showed that Bronopol is a mild irritant when applied in yellow soft paraffin (yellow petrolatum) at 0·25%. No evidence of sensitisation was seen in this study nor was there any suggestion of cross-sensitisation with any other substance, including formalin. The compound was applied as one of a battery of closed patch tests used in that clinic to screen the patients for a potential allergen. The patches were applied for 48 h and examined on the second and fourth days after the application. Of the 149 patients studied, three showed a slight erythema on the

Table VIII. The irritancy of Bronopol to human skin

| Base | Bronopol concentration % | Positive skin response | Degree of reaction |
|---|---|---|---|
| Soft Paraffin | 0 | 0/10 | — |
| | 0·5 | 0/10 | — |
| | 1 | 2/10 | both slight erythema |
| | 2 | 4/10 | all moderate erythema |
| Aqueous buffer pH 5·5 | 0 | 0/10 | — |
| | 0·50 | 0/10 | — |
| | 0·1 | 0/10 | — |
| | 0·25 | 1/10 | slight erythema |

second day which had faded by the fourth day, and one a moderate erythema on the second day; this patient did not return for the second examination.

Marzulli and Maibach (23, 24) have studied the contact sensitisation in man of a number of commonly used biocides; and have concluded that, under the conditions of a closed patch test, Bronopol in yellow soft paraffin was a potential sensitizer. The challenge concentration in these studies was 2·5% which according to these authors was a non-irritant concentration. However, the studies reported above are not consistent with this view. The patch tests carried out by Marzulli and Maibach showed a dose–response relationship, and since the response decreased very rapidly to zero at an induction concentration of 2% which is considerably greater than that used in formulations, the authors inferred that Bronopol may be safely used in cosmetic formulations. In a further study, Maibach (25), has confirmed that Bronopol was a direct irritant to human skin at concentrations greater than 1% under these conditions. A subsequent sensitisation test included 93 normal subjects who were induced with 10 applications of 5% Bronopol in yellow soft paraffin under an occlusive dressing over a period of 3 weeks. After a rest period of 2 weeks the subjects were challenged at 0·25% Bronopol in yellow soft paraffin at a different site. No evidence of contact sensitisation was observed.

## FORMULATION STUDIES

Bronopol has been in use for more than 10 years at a level of 0·01–0·02% or more in conventionally formulated shampoos based on sodium lauryl ether sulphates and alkanolamine alkyl sulphates with 2% or more of a foam-boosting alkanolamide. When a freshly prepared formulation is challenged with $1 \times 10^6$ pseudomonads per ml the bacterial count is reduced to $<10$ per ml within 24 h. The inclusion of protein-derived materials, e.g. 0·1–0·5% Crotein C (hydrolysed collagen, Croda Chemical Ltd) does not affect this result.

The use of Bronopol in the preservation of shampoos has been described by Bryce and Smart (14), Schuster (26) and in protein shampoos by Tuttle, Phares and Chiostri (27). Barnes and Denton (28) found Bronopol at 0·02% to be one of the most satisfactory preservatives against Gram-negative bacteria in a cream, suspension and solution in their capacity test.

Combinations of preservatives can be justified on several grounds, one of these being to increase the spectrum of antimicrobial activity. It is established that the antibacterial activity of Bronopol is greater than its antifungal activity and its spectrum can be increased by the addition of parabens, Parker (29). Proserpio (30) and Jacobs, Henry and Cotty (31) have considered the combination of Bronopol with other agents in cosmetics and oil-water emulsions.

### *Medicated Skin Cream*

Bronopol in an anhydrous base or in an aqueous formulation of low pH may have applications as an active antibacterial agent in skin care products. Experimental medicated skin creams containing Bronopol and Hexachlorophane BP showed activity on the skin against *Escherichia coli* whereas a cream without Bronopol did not. Although it is theoretically possible that synergism between these compounds is occurring, other experience suggests that this is unlikely. It is concluded therefore that Bronopol is exhibiting antibacterial activity *per se* in these formulations.

The composition of the cream was as follows:

| | % w/w |
|---|---|
| Hexachlorophane BP | 0·5 |
| Bronopol | 0·1 or 0·2 |
| Sorbitol syrup | 13·5 |
| Arlacel 186 (ICI United States Inc.) | 1·5 |
| Cosmolloid wax 70H (Astor Petrochemicals)* | 7·5 |
| Light mineral oil | 20 |
| Aqueous citrate buffer pH 4·5 | to 100 |

* The 70H grade is no longer available. Cosmolloid wax 70 grade has almost identical properties.

The forearms of eight subjects were washed, dried and swabbed with alcohol to remove the transient skin flora. The creams were applied in 0·05-g amounts to areas of 5×2·5 cm, and 0·025 ml of a 1 in 10 dilution in broth of an overnight culture of *Esch. coli* NCTC 5934 was applied on to each cream. After 30 min contact between cream and organism each area was swabbed with alginate swabs. The swabs were dissolved in 10 ml of quarter strength Ringer solution containing 1% of sodium hexametaphosphate and 1% of polysorbate 80. Aliquots (1 ml) of the swab diluents were plated in 'Oxoid' MacConkey agar No. 3 and plate counts were made after incubation for 48 h at 37°C. Results of a typical experiment are shown in *Table IX.*

**Table IX.** Antibacterial activity of medicated cream formulations on human skin. Viable organisms (per ml) from subjects (A–H) after contact time of 30 min

| | Subjects | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Cream | A | B | C | D | E | F | G | H |
| Base (no active agent) | + | + | + | + | + | + | + | + |
| Base+0·5% Hexachlorophane BP | + | + | + | + | + | + | + | + |
| Base+0·5% Hexachlorophane BP+ 0·2% Bronopol | 7 | 2 | 1 | 1 | 2 | 0 | 3 | 9 |
| Base+0·5% Hexachlorophane BP+ 0·1% Bronopol | 11 | + | + | 6 | 20 | 20 | 0 | 29 |

+ = uncountable numbers.

### *Alcoholic Deodorant Spray*

An experimental alcoholic deodorant spray formulation containing 0·12% w/w Bronopol was found to be stable after one-year's storage at room temperature and 37° in a lacquered tinplate can, and showed a broad spectrum of antibacterial activity.

Composition of the spray was as follows:

| | % w/v |
|---|---|
| Diethyl Phthalate BPC | 0·330 (v/v) |
| Propylene glycol | 0·167 |
| Denatured ethanol B grade 750P | 29·320 |
| Perfume | 0·083 |
| Bronopol | 0·100 |
| Propellant 11/12 | (1 : 1) to 100 |

This formulation was packed into internally lacquered tinplate cans soldered with solder 2/98 and fitted with a standard valve. The can fill was 128 g. Samples were then placed on storage test at room temperature and 37°C, and examined at regular intervals over a period of one year.

Chemical assays were carried out using an adaptation of the t.l.c. system described earlier. The results showed that no decomposition of the Bronopol had occurred. No corrosion of the container was observed.

Microbiological assessments were carried out by spraying the formulation on to a 13 mm Whatman A.A. disc and allowing to dry. The discs were then placed on to agar seeded with various test bacteria and *Candida albicans*. The zones of inhibition were recorded after 18 h at 37°C. The results obtained after one-year's storage are shown in *Table X*.

**Table X.** Antibacterial activity of Bronopol in an alcoholic spray formulation after one year storage at room temperatue and 37°C

| Test organism | Diameter of zone of inhibition (+13 mm disc) in mm | | |
|---|---|---|---|
| | Sample 1 (containing Bronopol initially at 0·12% w/w) | | Sample 2 (containing no Bronopol) |
| Temperature of storage | Room temperature | 37° | 37° |
| *Staph. aureus* 8452 | 29 | 30 | 13 |
| *Staph. aureus* FDA | 35 | 35 | 13 |
| *Staph. albus* NCTC 7944 | 28 | 29 | 13 |
| *Esch. coli* NCTC 5934 | 26 | 26 | 13 |
| *Proteus vulgaris* NCTC 4635 | 26 | 26 | 13 |
| *Ps. aeruginosa* 10S | 28 | 28 | 13 |
| *Candida albicans* 239 | 13 | 13 | 13 |

### *Personal Hygiene or Foot Spray*

An experimental hygiene spray formulation containing Bronopol was found to be stable in a lacquered aluminium can and showed more antibacterial activity than a similar formulation containing chlorhexidine, even after 3 months storage at elevated temperature.

The following formulation was chosen to represent a typical hygiene spray:

| | | % w/w |
|---|---|---|
| Talc 399 (Whittaker, Clark and Daniels Inc.) | | 0·500 |
| Bronopol | | 0·075 |
| Perfume | | 0·100 |
| Propellant 11 (I.C.I. Ltd) | | 39·325 |
| Propellant 12 (I.C.I. Ltd) | to | 100 |

Crystalline Bronopol was passed through a micro-mill to obtain the necessary reduction in particle size, the material subsequently passed through a 250 mesh screen sieve. The iron content of the Bronopol was 7 ppm and no evidence of discolouration was evident after 5 months storage at room temperature. Particle size analysis of the talc showed 98·7% less than 15 µm.

The above formulation was packed into an internally lacquered aluminium monobloc can fitted with a standard valve. The can fill was 64 g. Samples were placed on storage test at room temperature and 37°C, and were examined at regular intervals over a period of 3 months.

Chemical assays were carried out using an adaptation of the t.l.c. system described earlier. The results showed that no decomposition of Bronopol had occurred. No corrosion of the container was observed.

Microbiological assays were carried out using the method described for the alcoholic deodorant spray formulation. The results obtained after three months storage are shown in *Table XI.*

**Table XI.** Antibacterial activity of Bronopol and chlorhexidine in a hygiene spray formulation after 3 months storage at room temperature and 37°C

| Test organism | Diameter of zone inhibition (+13 mm disc) in mm | | | | | |
|---|---|---|---|---|---|---|
| | Sample 1 (containing Bronopol initially at 0·075% w/w) | | Sample 2 (containing chlorhexidine initially at 0·075% w/w) | | Sample 3 (containing neither biocide) | |
| Temperature of storage | Room temperature | 37° | Room temperature | 37° | Room temperature | 37° |
| *Staph. aureus* 8452 | 30 | 25 | 14 | 15 | 15 | 14 |
| *Staph. aureus* FDA | 32 | 22 | 15 | 15 | 13 | 13 |
| *Staph. albus* NCTC 7944 | 31 | 24 | 14 | 14 | 13 | 13 |
| *Esch. coli* NCTC 5934 | 21 | 21 | 13 | 13 | 13 | 13 |
| *Proteus vulgaris* NCTC 4635 | 23 | 21 | 13 | 13 | 13 | 13 |
| *Ps. aeruginosa* 10S | 26 | 22 | 13 | 13 | 13 | 13 |
| *Candida albicans* 239 | 13 | 13 | 16 | 16 | 13 | 13 |

Further microbiological tests were carried out with samples which had been stored at room temperature and 37°C for 6 months. Although these tests were only qualitative, a similar pattern of activity to that observed after 3 months was obtained.

## CONCLUSIONS

Bronopol has been shown to possess a wide spectrum of antibacterial activity. Its activity against Gram-negative organisms, particularly *Ps. aeruginosa,* is greater than that of most other antibacterial and preservative agents.

Bronopol is most stable under acid conditions, although it demonstrates high antibacterial activity over a wide pH range. The mode of decomposition has been studied in detail, and a number of the decomposition products identified. The assay methods described are capable of estimating Bronopol in many formulations at its normally used levels, the sensitivity of the methods is dependent upon the nature of the formulations; in certain cases levels as low as 5 ppm can be assayed.

Bronopol is generally used as a preservative in formulations at levels between 0·01 and 0·1%. Animal toxicity studies and human patch tests have demonstrated the safety of Bronopol when used at these concentrations. In particular, no evidence of human skin sensitisation has been obtained at these levels.

Bronopol has been shown to be an effective antibacterial agent in a range of formulations including shampoos, skin creams and sprays and bath products. Many of the ingredients used in such products have been shown to have little or no effect on the antibacterial activity of Bronopol, although compounds containing sulphydryl groups are antagonistic to its activity.

## ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of the following staff of The Boots Company Limited in the production of the data included: Dr D. F. Spooner (microbiology); Mr E. L. Crampton (chemistry); Mr D. A. Elvidge, the late Mr C. Vickers, and Mr J. S Wragg (analysis); Dr P. C. Risdall and Miss M. M. Sutton (toxicology); and Mr K. G. Jackson and Dr D. P. Stokes (formulation).

In addition, the authors wish to thank Dr C. D. Calnan, Institute of Dermatology, University of London; and Professor H. I. Maibach, Department of Dermatology, University of California for their help in the completion of the human skin studies.

## REFERENCES

1 Hodge, E. B., Dawkins, J. R. and Kropp, E. A new series of antifungal compounds. *J. Am. Pharm. Ass., Sci. Ed.* **43** 501 (1954).

2 Zsolnai, T. Versuche zur Entdeckung neuer Fungistatika. II. Nitro-verbindungen. *Biochem. Pharmac.* **5** 287 (1961).

3 Croshaw, Betty, Groves, M. J. and Lessel, B. Some properties of Bronopol, a new antimicrobial agent active against *Pseudomonas aeruginosa*. *J. Pharm. Pharmac.* **16** Suppl., 127T (1964).

4 Clark, N. G., Croshaw, Betty, Legetter, B. E. and Spooner, D. F. Synthesis and antimicrobial activity of aliphatic nitro compounds. *J. Med. Chem.* **17** 977 (1974).

5 Morse, L. J. and Schonbeck, L. E. Hand lotions, a potential nosocomial hazard. *New. Engl. J. Med.* **278** 376 (1968).

6 Sykes G. and Smart, R. Preservation of preparations for application to the skin. *Am. Perfum. Cosmet.* **84** 45 (1969).

7 Smart, R. and Spooner, D. F. Microbiological spoilage in pharmaceuticals and cosmetics. *J. Soc. Cosmet. Chem.* **23** 721 (1972).

8 Malcolm, S. A. and Woodroffe, R. C. S. The relationship between water-borne bacteria and shampoo spoilage. *J. Soc. Cosmet. Chem.* **26** 277 (1975).

9 Tenenbaum, S. The significance of pseudomonads in cosmetic products. *Am. Perfum. Cosmet.* **86** 47 (1971).

10 Thomas, M. J. and Majors, P. A. Animal and human microbiological safety testing of cosmetic products. *J. Soc. Cosmet Chem.* **24** 135 (1973).

11 Marples, R. R. and Kligman, A. M. Methods for evaluating topical antibacterial agents on human skin. *Antimicrob. Agents Chemother.* **5** 323 (1974).

12 Onoda, T. and Saito, H. Influence of a new antibacterial agent, Bronopol, upon the growth of cultured cells. *Chemotherapy* (*Tokyo*) **22** 196 (1974).

13 Hale, L. J. and Inkley, G. W. A semiautomatic device for multiple inoculation of agar plates. *Lab. Pract.* **14** 452 (1965).

14 Bryce, D. M. and Smart, R. The preservation of shampoos. *J. Soc. Cosmet. Chem.* **16** 187 (1965).

15 Brown, M. R. W. Turbidimetric method for the rapid evaluation of antimicrobial agents – Inactivation of preservatives by non-ionic agents. *J. Soc. Cosmet. Chem.* **17** 185 (1966).

16 Stretton, R. H. and Manson, T. W. Some aspects of the mode of action of the antibacterial compound Bronopol (2-bromo-2-nitropropane-1,3-diol). *J. appl. Bact.* **36** 61 (1973).

17 King, M. B., Knox, R. and Woodroffe, R. C. S. Investigation of antituberculous substances – an agar diffusion method using *Mycobacterium smegmatis*. *Lancet* **i** 573 (1953).

18 Kabay, A. Rapid quantitative microbiological assay of antibiotics and chemical preservatives of a non-antibiotic nature. *Appl. Microbiol.* **22** 752 (1971).

19 Moore, D. H., Chasseaud, L. F., Lewis, J. D., Risdall, P. C. and Crampton, E. L. The metabolism of the antibacterial agent Bronopol (2-bromo-2-nitropropane-1,3-diol) given orally to rats and dogs. *Fd. Cosmet. Toxicol.* **14** 183 (1976).

20 Moore, D. H., Chasseaud, L. F., Bucke, D. and Risdall, P. C. The percutaneous absorption and disposition of the antibacterial agent Bronopol in rats and rabbits. *Fd. Cosmet. Toxicol.* **14** 189 (1976).

21 Noakes, D. N. and Sanderson, D. M. A method for determining the dermal toxicity of pesticides. *Brit. J. Indust. Med.* **26** 59 (1969).

22 Stevens, M. A. Use of the albino guinea-pig to detect the skin sensitizing ability of chemicals. *Brit. J. Ind. Med.* **24** 189 (1967).

23 Marzulli, F. N. and Maibach, H. I. Antimicrobials: experimental contact sensitization in man. *J. Soc. Cosmet. Chem.* **24** 399 (1973).

24 Marzulli, F. N. and Maibach, H. I. The use of graded concentrations in studying skin sensitizers: experimental contact sensitization in man. *Fd. Cosmet. Toxicol.* **12** 219 (1974).

25 Maibach, H. I. Dermal sensitization potential of 2-bromo-2-nitropropane-1,3-diol (Bronopol). *Contact Dermatitis* **3** 99 (1977).

26 Schuster, G. Die Conservierung von Shampoos und Schaumbademitteln. *Seifen-Öle-Fette-Wachse* **99** 489 (1973).

27 Tuttle, E., Phares, C. and Chiostri, R. F. Preservation of protein solutions with 2-bromo-2-nitro-1,3-propanediol (Bronopol). *Am. Perfum. Cosmet.* **85** 87 (1970).

28 Barnes, M. and Denton, G. W. Capacity tests for the evaluation of preservatives in formulations. *Soap, Perfum. Cosm.* **42** 729 (1969).

29 Parker, M. S. Some aspects of the use of preservatives in combination. *Soap, Perfum. Cosm.* **46** 223 (1973).

30 Proserpio, G. Protection des cosmétiques par des mélanges synergiques de préservateurs à dosage microbiocide. *Parfum. Cosmet. Savons* **2** 305 (1972).

31 Jacobs, G., Henry, S. M. and Cotty, V. F. The influence of pH, emulsifier, and accelerated ageing upon preservative requirements of o/w emulsions. *J. Soc. Cosmet. Chem.* **26** 105 (1975).