

**Observations of the effects of
formaldehyde on cockroaches and their flora: II. Prolonged
survival of cockroaches drinking formaldehyde
or glutaraldehyde solutions**

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SUMMARY

Adult cockroaches were found to survive up to 22 weeks when provided with 1% Formalin (0.4% formaldehyde) in lieu of drinking water. Given 4% Formalin or 2% glutaraldehyde they survived up to 41 days. During the experiments eggs were laid and hatched and the offspring continued to grow. Combined with surface disinfection, this may hold out a simple method of rendering adult cockroaches gnotobiotic or even axenic.

INTRODUCTION

From time to time formaldehyde fumigation of hospital wards or experimental animal rooms is called for following high risk bacterial or viral disease in previous occupants. Great faith is usually placed in the efficacy of this procedure and it is commonly believed that no living organisms could survive in the customary concentrations of this highly toxic vapour.

In a previous paper (Bartzokas, McCarthy, Shackleton & Baker, 1978) we reported that cockroaches could tolerate formaldehyde vapour fumigation generated by double the quantities of reagents and for four times as long as that recommended for routine (Martindale, 1972) or smallpox (D.H.S.S., 1975) disinfection. We also reported that vaccinia virus fed to those insects before fumigation could often be recovered from the faeces afterwards.

In similar experiments we have occasionally been surprised to find that insects which had obviously consumed large quantities of live virus were not excreting virus after fumigation. The possibility arose that the timing of the humidification process which normally precedes fumigation might be critical. When this was done in such a way that condensation of water droplets occurred on the cockroaches' polystyrene box they had the opportunity to drink fluid in which formaldehyde gas had dissolved. We suspected that this might not be toxic to the cockroaches and that this might have accounted for the negative virus cultures from some experiments.

In this paper we report the effect on cockroaches of substituting formaldehyde or glutaraldehyde solutions for drinking water.

Table 1. *Number of cockroaches surviving 1% Formalin administration*

Weeks	1	2	3	4	5	6	7	8	9	10	11
Cockroaches surviving	11	10	9	9	8	6	5	5	5	4	4
Weeks	12	13	14	15	16	17	18	19	20	21	22
Cockroaches surviving	4	4	4	4	3	3	3	3	3	2	1

MATERIALS AND METHODS

The cockroaches used were adult *Periplaneta americana* (L.) kindly supplied by Professor C. J. Duncan of the Department of Zoology, University of Liverpool, or by a commercial supplier. They were housed in naturally ventilated polystyrene containers and were kept at room temperature (*ca.* 20 °C) in the laboratory with no special humidity control. The insects were fed only with crumbled dry mouse diet pellets. They were deprived of drinking water for 3 days before formaldehyde treatment. Solutions of formaldehyde at various strengths were made by diluting commercial 40% Formalin (Formaldehyde Solution (Methanal) 40% (w/v) May & Baker, Dagenham) with distilled water to give concentrations of 10 (i.e. 4% (w/v) formaldehyde), 7, 6, 5, 4 and 1%. As a preliminary to long-term studies, each strength was offered to small groups of cockroaches to obtain some estimate of concentrations likely to be imbibed. From the indications thus gained, a group of 11 was provided with a 4% dilution of Formalin (i.e. 1.6% (w/v) formaldehyde) as the only fluid. This was available *ad libitum* and was replaced daily or as required. In a second experiment an additional group of cockroaches was included supplied with glutaraldehyde as a 2% aqueous solution buffered at pH 7.5–8.5 (Cidex), in accordance with the manufacturer's instructions (Arbrook, Division of Ethicon Ltd., Bankhead Avenue, Sighthill, Edinburgh).

RESULTS

Groups of cockroaches offered 10 or 7% dilutions either backed away without drinking or sampled the fluid but died within a few days. Most cockroaches offered 6 or 5% dilutions drank moderately but sometimes appeared to regurgitate and/or passed diarrhoeic faeces within an hour and died within 48 h. The groups of cockroaches offered concentrations of 4% or less drank with little hesitation although initially they sometimes became agitated. On this basis a group of 11 was started on a regime of 4% Formalin in lieu of drinking water as the presumed highest tolerable concentration. These insects were seen to drink large quantities and to feed normally on the crumbled mouse pellets for the duration of the experiments. Table 1 shows that, although there were no immediate deaths, insects died sporadically over the succeeding weeks. However, four cockroaches survived for 15 weeks in apparent health and three for 20 weeks. During this 4-month period eggs were laid and hatched and the offspring continued to grow. The experiment was terminated at 22 weeks when there was only one original survivor.

Table 2. Number of cockroaches surviving various strengths of aldehyde administration

Days	On Formalin						On
	1%	4%	5%	6%	7%	10%	glutar- aldehyde 2%
0	15	15	5	5	5	5	15
1	14	14	5	5	5	5	15
2	14	10	1	5	5	None	15
3	12	7	1	5	4		15
4	12	7	1	4	3		15
5	11	6	None	3	2		15
6	11	5		2	None		15
7	9	3		1			15
8	7	1		1			14
9	7	1		1			13
10	5	1		1			11
11	3	1		1			9
12	3	1		None			8
13	3	1					7
14	3	1					6
15-22	2	1					5
23	2	1					4
24	2	1					2
25-41	1	1					1

The second experiment used the same concentrations of formaldehyde with other groups of cockroaches. An extra group was provided with 2% glutaraldehyde (Table 2). Although deaths occurred at an earlier stage in this experiment, there was evidence of substantial tolerance of formaldehyde. Of the 15 cockroaches in the three groups which had been provided with 5 or 6 or 7% Formalin, eight survived for 4 days and one for 11 days. Of the group of 15 which was given 4% Formalin three survived for a week, while nine out of the group of 15 given 1% Formalin survived for this period. A surprising result was that all 15 of the group offered 2% glutaraldehyde survived the 7th day and one till the termination of the experiment on the 41st day.

DISCUSSION

It is known to professional entomologists that formaldehyde is not an effective insecticide for cockroaches (Walker, 1964) but, to our knowledge, there have been no long-term studies of the continued administration of formaldehyde or glutaraldehyde solutions as the sole source of free water. We were, therefore, surprised to find that concentrations as high as 4% of Formalin could be tolerated for up to 4 months; an equally surprising finding was the apparent lack of acute toxicity of 2% glutaraldehyde. The occasional failure to recover vaccinia virus from faeces of infected cockroaches after formalin fumigation reported elsewhere (Bartzokas *et al.* 1978) may well be attributable to the insects having imbibed condensate containing dissolved formaldehyde.

Formaldehyde 4% solution (10% Formalin) is biocidal for viruses, bacteria and

bacterial spores (Cruickshank, Duguid, Marmion & Swain, 1975). However, concentrations as low as 0.04–1% formaldehyde (0.1–2.5% Formalin) are effectively used in disinfecting bacterial suspensions for vaccines or agglutination tests (Cruickshank *et al.* 1975) while 0.025% Formalin (Salk *et al.* 1955) is used in the preparation of Salk vaccine.

It can be seen that there was some variation in the time for which two groups of cockroaches tolerated 4% Formalin. Cockroaches are not, in our experience, as predictable in their life expectancy as are mice for example and we have noted such variations in survival time in other experiments. Nevertheless it seems a significant observation that even a small proportion could tolerate a concentration of formaldehyde which is 4/10 of the maximum recommended for disinfection and a concentration of glutaraldehyde equal to the maximum recommended for any purpose by Martindale's *Extra Pharmacopoea* (1972). This 2% concentration of glutaraldehyde contains approximately the same number of aldehyde groups as 3% Formalin.

Although the external application of high concentrations of formaldehyde vapour – approximately 1–2 mg/l of air – was not effective in rendering the external surface of vaccinia virus-infected cockroaches virus-free, these creatures readily tolerated the external application of 2% glutaraldehyde (Bartzokas *et al.* 1978*a*). This effectively rendered the surface virus-free and, combined with formaldehyde or glutaraldehyde as drinking water, may hold promise of achieving either controlled (gnotobiotic) or even total (axenic) sterility of adult cockroaches.

Previous workers have reported the production of gnotobiotic colonies of cockroaches by treating the oothecae with disinfectants such as alcohol, mercuric chloride or sodium hypochlorite (Benschoter & Wrenn, 1972; Clayton, 1959; Gier, 1947; Wollman, 1926) but we know of no attempts to render adult cockroaches bacteriologically sterile. It is not possible to speculate usefully on the possible effect on the so-called 'intra-cellular bacteroids' (Gier, 1947) because it is not known how effectively the formaldehyde penetrated the soma of the cockroaches in our experiments. We were, however, able to detect chemically the presence of Formalin in the faeces of insects on treatment and in short-term studies reported elsewhere (Bartzokas *et al.* 1978*b*), using 1% Formalin as drinking water, neither bacteria nor fungi could be detected in faeces after 5 days of this treatment after careful neutralization of residual formaldehyde.

Formalin was at one time regarded as a universal biocide but the finding that slow viruses such as Creutzfeldt-Jakob disease virus will withstand 10% Formalin for months (Gajdusek & Gibbs, 1976) has brought the totality of this view into doubt. We have not investigated the mechanisms underlying the extraordinary tolerance of cockroaches to formaldehyde and glutaraldehyde but it would seem to apply not only to exposure to high concentrations of vapour but also to ingestion of 4% Formalin over long periods and draws attention yet again to the ruggedness of this insect.

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