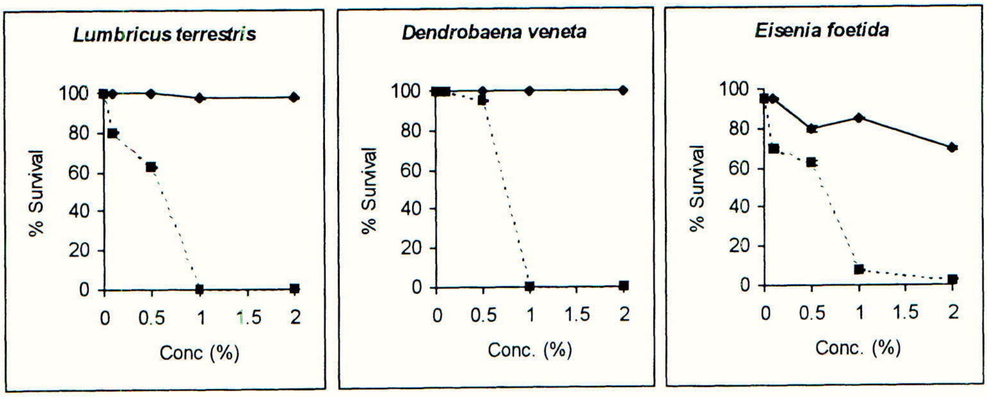

Figure 1. Survival of three species of earthworm; *Lumbricus terrestris*, *Dendrobaena veneta* and *Eisenia foetida*; after one week in soil/compost wetted with solutions of metaldehyde (♦) or caffeine (■) at concentrations of 0 (control), 0.01%, 0.1%, 0.5%, 1% and 2%.

Slug mortality

Analysis of slug survival time revealed differences in treatment (Kruskal-Wallace test, $P<0.001$). A Mann-Whitney *U*-test showed that slugs exposed to both metaldehyde doses had significantly lower survival rates than slugs exposed to both caffeine doses and the control treatment ($P<0.001$) (Fig. 2a). No significant differences were found between the two caffeine doses and control treatments (Fig. 2a). In addition, no significant differences were found between the two metaldehyde treatments (Fig. 2a).

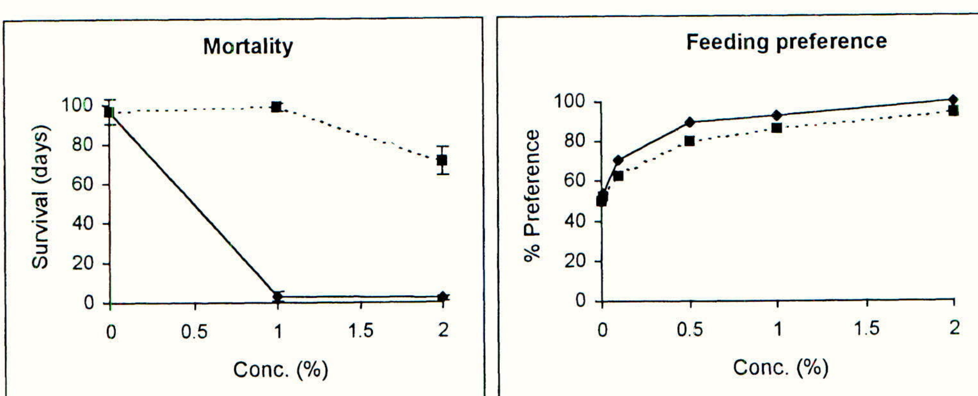

Figure 2. Relationship between the concentration of metaldehyde (♦) or caffeine (■), and (A) survival of slugs (*Arion subfuscus)*, and (B) feeding preference for untreated over treated leaf discs.

Slug feeding preference

The percentage of each leaf disc eaten (treated and untreated) in each petri dish was calculated to show the percentage of the untreated leaf disc eaten in comparison to the treated disc, called the % preference. ANOVA of this showed a significant effect of treatment ($P<0.001$). Feeding on leaf discs treated with the four highest doses of metaldehyde and caffeine was significantly reduced in comparison to the untreated discs in these treatments ($P<0.001$) (Fig. 2b). No significant differences were found between the lowest dose of both metaldehyde and caffeine in comparison with the control (Fig. 2b). Feeding on both metaldehyde- and caffeine-treated discs was also significantly reduced with the increase of each of the doses tested, except between 0.5% and 1% metaldehyde (Fig. 2b).

DISCUSSION

In slug mortality experiments we found that the survival of slugs (*A. subfuscus*) was reduced by metaldehyde, but not caffeine. This is in contrast to the findings of Hollingsworth *et al.* (2003), who found in treatment of 1% and 2% caffeine the number of dead slugs was significantly greater than the control after 48 hours. Hollingsworth *et al.* (2003) did not test the effect of metaldehyde solutions on the mortality of slugs, but our results support the findings of Hata *et al.* (1997), who also found that metaldehyde liquid formulations significantly increased slug mortality. In terms of environmental load, it is not possible to ascertain exactly how these experimental treatments relate to field application rates. However, if we assume that 1 cm of irrigation would be sufficient to replicate these findings, a 1% caffeine treatment would equate to 100 kg of caffeine being applied ha^{-1}. This conservative estimate is far greater than the current European field application rates of molluscicidal bait pellets of 0.48 kg active ingredient (a.i.) ha^{-1} for metaldehyde and 0.22 kg a.i. ha^{-1} for methiocarb. In slug feeding preference experiments, we found both metaldehyde and caffeine significantly reduced feeding of treated leaf discs at doses of 0.1%, 0.5%, 1% and 2%, but not at the lowest dose of 0.01%. Our results also show an increase in repellency with increasing dose of caffeine and metaldehyde over 0.1%. This is in agreement with the Hollingsworth *et al.* (2003) no-choice test on caffeine, but not the choice test. From these experiments a dose of 1% or 2% caffeine would be required for acceptable crop protection in the field. To determine precisely how this treatment would equate to normal agronomic practice is again difficult. However, in order to estimate a best guess, we weighed Chinese cabbage leaves before and after dipping in water then calculated the mean amount of water per unit area (10.67 mg cm^{-2}). Water would need to be applied at 1067 litres/ha to achieve this level of area coverage. A 2% caffeine spray applied at this rate would equate to over 21 kg/ha. In addition, we found 1% and 2% caffeine solutions caused leaf discs to turn yellow and decompose rapidly (data not shown). This is in agreement with Hollingsworth *et.al* (2002, 2003) who found leaf yellowing in ferns, bromeliads, cabbage and lettuce at concentration of between 0.5 and 2% caffeine. In contrast, all concentrations metaldehyde solutions tested did not cause leaf yellowing or premature decomposition. This is in agreement with Simms *et al*, (2002), who found metaldehyde seed treatments were not phytotoxic at any level tested on oilseed rape.

We found caffeine to be toxic to all earthworm species tested at low doses, with two species (*L. terrestris* and *E. foetida*) being significantly affected by all doses tested and the other (*D. veneta*) being affected at the highest two doses. In contrast only one species of earthworm showed any detrimental signs to the presence of metaldehyde at two of the doses tested and the effects of metaldehyde were relatively small and significantly less than those of caffeine at the two highest doses tested.

We conclude that caffeine, used as a molluscicide, would be more toxic to a wider range of organisms and would need to be applied at higher rates, than the most commonly used molluscicide, metaldehyde. We therefore suggest that caffeine has no environmental or efficacy benefits over metaldehyde. It is clear that the scientific community and public perception of 'natural products' being less detrimental to the environment than pesticides are not always accurate and perhaps the pesticide industry needs to work harder to improve its image.

ACKNOWLEDGEMENTS

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A trial on the use of biological aerated filter (BAF) technology for combating the 'snail problem'

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ABSTRACT

Since the late 1990s Thames Water UK has experienced snail infestations within a handful of its secondary plastic mineral trickling filters (PMTFs), i.e., filters whose prime purpose is to remove ammonia. At these sites, snails consume biofilm at such a rate as to completely wipe out any autotrophy nitrifying bacteria populations living within the filters. As a consequence, these sites would have failed their ammonia discharge consents had remedial action not been taken.

Biological aerated filters (BAF) represent an alterative wastewater treatment process. They contain stationary media granules to which microbial growth becomes attached. The granules themselves are submerged during normal operation in aerated wastewater. This paper outlines the results of a study designed to determine whether or not a pilot-scale BAF unit was as susceptible to snail infestation as a plastic media trickling filter.

At loadings designed to achieve effluent standards equivalent to a secondary PMTF, it was found that the BAF unit was unable to support a snail population of any size. The robustness of the BAF unit was attributed to the efficient packing of the media and the consequent difficulty for snails to move and feed within the unit.

INTRODUCTION

Berkhamsted Sewage Treatment Works (STW) is an unusual site consisting of primary and secondary plastic media filters but without any form of primary sedimentation. Since the late 1990s there has been an invasion of snails in the secondary filters (Dussart, 2000). The snails in question are the common British "wandering pond snail" *Lymnaea peregra*, which lays eggs in early spring and early autumn and lives for up to 2.5 years. Thus one adult can routinely produce 4-5 generations of offspring (Dussart, 1976; 1979).

The snails eat so much biofilm that the filters fail to remove ammonia. As an internal solution, the filters have been dosed with copper sulphate. The technique kills the snails but experience has shown it is only a matter of time before they return.

Where snails could be present, Thames Water recommends that plastic media filters should not be used for secondary treatment. It is therefore necessary to start investigating new secondary treatment options. One such process to be considered is the Biological Aerated Filter (BAF).

BAF represents an alternative secondary treatment process, containing stationary media granules to which microbial growth becomes attached. The granules themselves are submerged during normal operation in aerated wastewater.

The BAF process offers a number of advantages: the attached biofilm on an inert granular medium in BAF allows for a much higher concentration of active biomass than an activated sludge system, so that the size of reactor can be reduced. In addition, suspended solids (SS) in the influent can be captured physically by the medium, eliminating the requirement for separate secondary clarification. Overall, these advantages result in a space-saving layout that uses only one-third the footprint space of an activated sludge process.

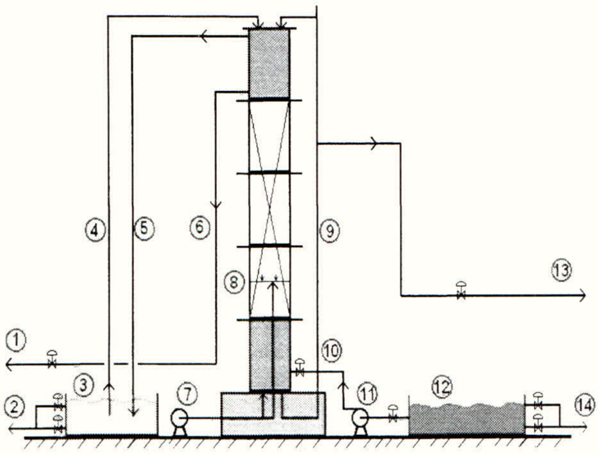
This paper outlines the results of a study designed to determine whether or not a pilot-scale BAF unit was as susceptible to snail infestation as a secondary plastic-medium trickling filter, and whether the BAF could therefore be used as an alternative technology in wastewater treatment.

EXPERIMENTAL PROCEDURE

Initially, the BAF pilot-scale column was operated to check whether it could remove ammonia (NH_3), biological oxygen demand (BOD_5) and SS efficiently, and to see if snails would naturally take up residence in the column (the column was fed by effluent from the primary humus tanks that had a small snail population). After a month or so, snails were deliberately fed into the column to determine whether or not the BAF unit was a favourable place for snails to live and breed.

BAF column

A pilot-scale BAF column was constructed by Bright Water UK (Fig. 1). It was 3 m in height and 40 cm in diameter. The major section of the column was constructed from clear PVC whilst the bottom and the top sections were opaque PVC. The feed came from the distribution chamber for the second stage plastic media trickling filters at a maximum flow rate of 4 l/min, and flowed up through the column. The media consisted of plastic beads 2-3mm in diameter, occupying the top two thirds of the column and held in place by a metal mesh at the top of the column. Process air was introduced into the packed media approximately 30 cm above the bottom, allowing the first 30 cm of the media to operate as a filter rather than as a biological process. The final effluent was collected in a tank, and the column was backwashed approximately three times a week.



1. To the underdrain tank, 2. To the underdrain tank, 3. Final effluent tank, 4. Backwashing water, 5. Final effluent flow, 6. Top dirty water flow, 7. Scour and process air compressor, 8. Process air supplier, 9. Recycle flow, 10. Feed into the column pipe, 11. Feed pump, 12. Feed (influent) tank, 13. Bottom desludge flow to the underdrain tank, 14. To the underdrain tank

Figure 1. A pilot-scale BAF column for the study

Sample collection and analysis

24-hour composite influent and effluent samples were collected 10 days after the start-up of the pilot plant, and then taken three times a week throughout the trial. The samples were analysed for suspended solid (SS), biological oxygen demand (BOD_5) , and ammonia (NH_3).

RESULTS AND DISCUSSION

During the first month of the study period, the BAF column proved to work efficiently in terms of removing NH_3, BOD_5, and SS. These removal efficiencies were equivalent to those that would be expected from a snail-free secondary stage PMTF. In addition, no snails were observed within the BAF column during the first month.

After the snails were fed into the column (approximately 2000 snails), performance did fall off for the first few days, but quickly returned to normal. One month following the introduction of the snails the media were removed and examined for snails – not a single live snail was found. This process was repeated with a fresh population of snails (approximately 2000 snails) but this time the media were examined after 21 days – again not a single live snail was found. Thus, it would appear that the BAF column is not a suitable environment in which snails can live and breed.

The concentrations of NH_3, BOD, and SS of influent and effluent samples are presented in Figures 2 to 4.

Figure 2 shows that the SS removal efficiency varied from 37.8% to 82.1%. The low efficiencies were due to operational problem.

The target upper 95%ile for the ammonia was 5 mg/l: the final effluent varied from 0.1 to 4.7 mg/litre, with the mean being 0.8 mg/litre. The efficiency of the column for NH_3 removal was between 48.8 and 98.4% (Figure 3). Note that the low removal efficiencies occurred at the beginning of the experiment before the BAF unit was properly seeded.

The effluent BOD concentration for the Berkhamsted STW should not exceed 15 mg/litre. As it can be seen from Figure 4, the removal efficiency of BOD with the BAF column was between 47.2% and 85.7 %, the average being 85%.

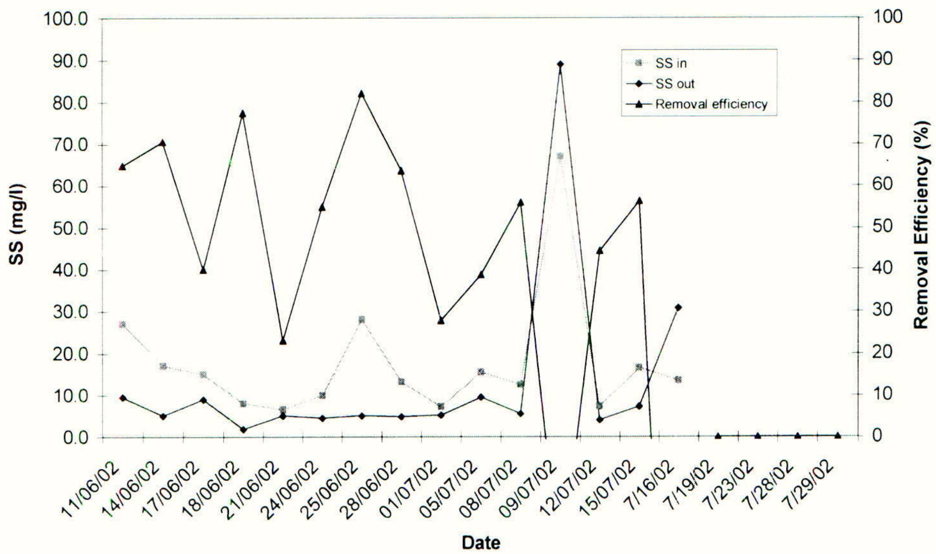


Figure 2. Suspended solids (SS) removal

The inability of the snails to live within the BAF and their ability to flourish in a PMTF can be understood in terms of the snails' capability to feed within the two process units. In a PMTF, the average void space is approximately 20mm and so the snails (approximately 5mm in size) are able to move around within the PMTF unimpeded. However in a BAF unit, the average pore size between the packed beads is approximately 2mm, and so the snails are unable to move around within the BAF unit. Since snails feed by moving and grazing on the biofilm, being unable to move means they are unable to feed and so die.

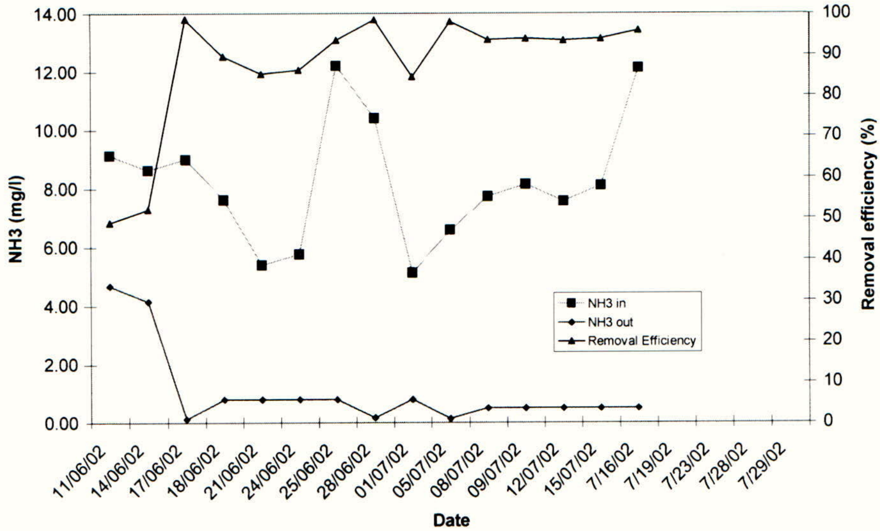


Figure 3. Ammonia (NH3) removal

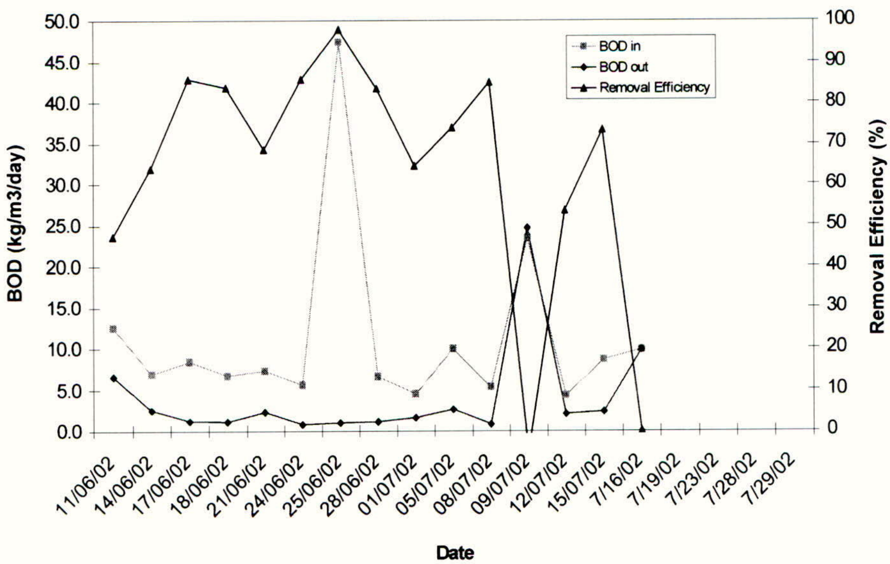


Figure 4. BOD removal

CONCLUSIONS

The environmental conditions at Berkhamsted STWs are such that the secondary plastic media trickling filters, a technology inherently prone to snail infestation, have indeed suffered from snail infestation. However, under the same environmental conditions, this study has shown that a pilot scale BAF unit not only meets the same effluent standards as a PMTF but is also unable to support a snail population of any size.

BAF technology can be considered an alternative secondary treatment process option for sites that could be prone to snail infestation.

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The environmental profile of metaldehyde

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ABSTRACT

Since 1937, the dry alcohol metaldehyde has been known to act as a molluscicide. Detailed studies have shown that the main effect on slugs is that the mucus cells are irreversibly damaged. Mucus cells are part of important physiological structures typical of molluscs. Metaldehyde is not phytotoxic and in no cases have negative effects been recorded on the carabid beetles *Poecilus cupreus*, *Carabus granulatus*, *Pterostichus melanarius*, *Harpalus rufipes*, the staphylinid beetle *Aleochara bilineata*, the honey bee (*Apis mellifica*), the aphid parasitoid *Aphidius rhopalosiphi* and the predatory mite *Typhlodromus pyri*. No adverse effects were found on three earthworm species (*Lumbricus terrestris*, *Allolobophora chloroti* and *Eisenia fetida*). Studies with wild living mammals, hedgehogs (*Erinaceus europaeus*) fed with metaldehyde-contaminated slugs and wood mice (*Apodemus sylvaticus*) exposed to metaldehyde slug pellets did not show any signs of disturbance. No adverse effects on tilapia (*Tilapia mossambicus*), carp, milkfish (*Chanos chanos*) and on Crustacea were found in aquatic systems. Metaldehyde does not show any tendency to accumulate in soils, water bodies, plants and mammals. Under natural conditions it completely degrades to CO_2 and H_2O.

THE ACTIVE INGREDIENT METALDEHYDE

The first report of metaldehyde as a slug control agent was by Gimingham & Newton (1937), after which, it attracted the attention of agricultural research as a molluscicide. The first commercial formulations were offered to farmers, vegetable and ornamental growers. Metaldehyde, first discovered by von Liebig in 1835, is the cyclic tetramer of acetaldehyde forming tetragonal prisms (Figure 1).

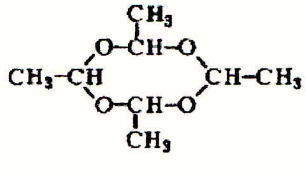

Figure 1. Chemical structure of metaldehyde

Metaldehyde is a pure hydrocarbon that degrades to acetaldehyde, then to acetic acid and thereafter into water and carbon dioxide (CO_2). Acetaldehyde is a naturally occurring substance and an intermediate in the degradation chain of ethanol in mammals. The mode of action of metaldehyde on slugs has been investigated by Triebskorn (1989), Triebskorn & Ebert (1989), Triebskorn & Schweizer (1990) and Triebskorn, *et al.* (1998). In these studies, the mucus cells of slugs, typical of land molluscs, were irreversibly destroyed. Metaldehyde acts quickly in slugs or snails, severe altering and destroying the ultrastructure of mucocytes independent of factors like low temperatures and high precipitation rates.

DEGRADATION AND FATE OF METALDEHYDE IN THE ENVIRONMENT

In aerobic topsoils, metaldehyde degraded completely within a few days. In average German agricultural soils, a DT_{50} of 5.3 to 9.9 days were observed (Lonza proprietary data). Similar results were found for water sediments in moderate temperature conditions (Figure 2). Metaldehyde was completely degraded with a DT_{50} of about 12 days. Its only metabolite, acetaldehyde, was formed transiently and finally mineralised to CO_2. The original carbon from metaldehyde was recovered as carbon dioxide.

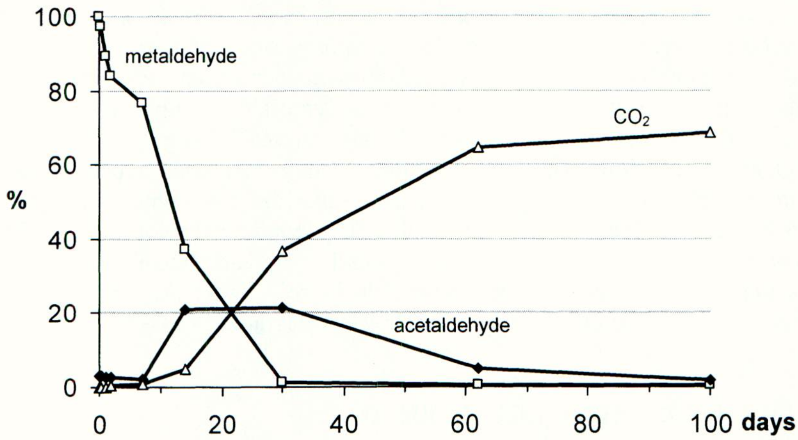

Figure 2: Degradation of metaldehyde in water sediment over a 100 day period at moderate temperatures (Lonza proprietary data).

More rapid degradation of metaldehyde has been reported by Calumpang, *et al.* (1995) in rice paddies in the Philippines. The maximum concentration measured in the water body was 1.58 mg/l but it fell below the detection limit 9 days after treatment. The calculated half-life time was 0.27 days and the metaldehyde degraded completely in the soil. It had a peak concentration of 0.127 mg per kg soil, but 22 days after treatment, had fallen below detection limits The authors concluded that metaldehyde did not cause persistent residues in the various components of a rice paddy ecosystem. Similar results were found by Coloso, *et al.* (1998) where metaldehyde content constantly fell within 15 day to 1 % of its original concentration in the sediment of fish ponds. In the water, the metaldehyde content decreased to 16 % of the maximum concentration in the same time range. For higher vertebrates, there is no risk for accumulation in the organism if metaldehyde is ingested at low doses. Metaldehyde gets metabolised in the same way as ethanol, a substance that naturally occurs in small but clearly measurable amounts in almost any ripe fruit.

EFFECT OF METALDEHDE ON NON TARGET ORGANISMS

In recent decades, the knowledge of the sometimes highly sensitive interactions within our agro-ecosytems has increased. With this growing awareness about the agronomic value of beneficial organisms has come a need to know the effects of agrochemicals on these same organisms.

Earthworms

Earthworms are the most obviously beneficial organisms to be exposed to the effects of slug control measures. Slugs and earthworms share the same habitats. For small slugs, earthworm burrows may serve as refugees to survive cold or dry periods in deeper soil layers. Molluscicides are applied in the form of pellets. Earthworms collect algae or leaf litter to bring into their burrows from the soil surface. Earthworms in treated fields come into direct contact with slug pellets. Because earthworms are important for soil health, they should not be endangered by slug treatment. Bieri, *et al.* (1989), Fayolle & Stawietcky (1990) and Högger, *et al.* (1992) did not find any behavioural change in *Lumbricus terrestris* or *Allolobophora chlorotica* exposed to metaldehyde slug pellets in the laboratory. Although worms may have ingested considerable doses of metaldehyde, Bieri, *et al.* (1989) found no adverse effects.

The NOEL of *Eisenia fetida* is more than 1000 mg metaldehyde per kg soil, measured according to OECD procedure Nr. 207.

Beneficial arthropods

The other important group of soil dwelling animals are the predatory arthropods. In semi-field trials, Büchs, *et al.* (1989, 1990) investigated the effects of slug pellets on *Poecilus cupreus*, *Carabus granulatus*, *Pterostichus melanarius* and *Harpalus rufipes* of the Carabid family in the laboratory in semi-field tests as well as in the field. In the laboratory, *Carabus granulatus* showed sensitivity against metaldehyde pellets, whereas in an extended laboratory test and in a semi-field test, the natural mortality clearly exceeded that found in the laboratory plots, with Metaldehyde pellets at application rates of 4.5 to 5.3 times the rate recommended by the pellet producers.

The effect of metaldehyde slug pellets on the predatory roof beetle *Aleochara bilineata* was investigated by Samsøe-Petersen, *et al.* (1992) in the laboratory. *Aleochara bilineata* females were placed in jars of a diameter of 3.5 cm with at least one slug pellet. This gave an over-dosage of 30 to 33 times that of the field rate. No mortality due to the metaldehyde slug pellets was observed and the number of eggs laid per female did not differ compared to the control.

In a field test where each plot was fenced by iron sheeting, the effect of scattered slug pellets on soil-dwelling arthropods was recorded by Bieri, *et al.* (1989). Some plots were treated with approximately 4 times the field rate of metaldehyde slug pellets. The numbers of arthropods was measured with pitfall traps placed in the centre of each plot. In no case could an effect on spiders, ants, millipedes, staphylinid and carabid beetles be observed in the areas where metaldehyde slug pellets were applied.

In different laboratory tests, no adverse effects on beneficial arthropods were observed. After direct contact or oral exposure to metaldehyde, Honey bees did not show any adverse reactions (Lonza proprietary data). The predatory mite *Typhlodromus pyri* and the aphid parasitoid *Aphidius rhopalosiphi* did not show any signs of irritation when exposed on a Metaldehyde containing surface (Lonza proprietary data).

Wild vertebrates

Hedgehogs (*Erinaceus europaeus* L.) are protected animals in Europe. They are beneficial and are known to be slug eaters. Many people take the presence of hedgehogs as an indication of a sound biotope. There is therefore a justified concern that hedgehogs, as slug predators, could be affected by slug pellets. At the German Research Station of Agriculture and Forestry in Münster, Gemmeke (1995) exposed dead slugs which had ingested metaldehyde slug pellets to six hungry hedgehogs. This experiment took place in specially equipped cages and another six animals were used as controls. No aversion behaviour was observed even though the animals ate almost all, or all, of the 200 slugs offered per cage. Gemmeke (1995) could not observe any effects or behavioural changes in hedgehogs which had consumed Metaldehyde-containing slugs. Schlatter, cited in Esser (1984), found that a dose of 500 mg / kg body weight did not cause problems to hedgehogs.

Tarrant, *et al.* (1990) studied the effect of metaldehyde slug pellets on a wood mouse (*Apodemus sylvaticus* L.) population in a newly-sown field of winter cereals. They found no evidence of exposure, since none of the wood mice analysed contained detectable residues of metaldehyde, nor were there adverse effects on individual wood mice at the individual or population level.

Several tests for studying the effect on bird species have indicated that at recommended application rates, metaldehyde pellets are not attractive to birds.

Aquatic animals

For more than a decade metaldehyde formulations have been used against aquatic snail pests mainly in South East Asia. Cheng (1989) reported on the control of the introduced snail *Pomacea lineata* invading rice paddies in Taiwan. According to his studies, metaldehyde was quite selective for *P. lineata,* having no effect on freshwater shrimps and fishes. It is the only molluscicide allowed for use in Taiwan in ponds, irrigation ditches and other water systems whenever fish toxicity is a concern.

Tilapia and Carp exposed to paddy rice treatments with metaldehyde formulations did not show any mortality in directly exposed cages positioned in the treated area (Calumpang, *et al.* 1995).

In a study of juvenile Milkfish (*Chanos chanos*), Borlogan, *et al.* (1996) exposed fish in test basins to Metaldehyde formulations (10 % active ingredient) with application rates of 0, 25, 50, 75, 100, 125, 150 and 175 kg/ha. No mortality could be observed within seven days for fish at 1 to 2 g live-weight.

Investigations on metaldehyde toxicity for the fish species *Tilapia mossambicus*, the shrimp species *Penaues monodon* and *Metapenaues ensis*, the crab *Scylla serrata* and the small crustacean *Artemia salina* by Coloso & Borlogan (unpublished data) have showed that Metaldehyde applications present no risk to these animals.

PHYTOTOXICITY

Metaldehyde has been used for many years in all kind of crops and all over the world. In all these years, there have been no reports of phytotoxicity. Ester & Nijënstein (1995) investigated the effect of Metaldehyde on the growth of the perennial ryegrass (*Lolium perenne*) after seed treatment. Even at rates of 320 g active ingredient per kg seed, no phytotoxicity could be observed for either the seedlings or for plant growth.

CONCLUSIONS

An ideal pest control agent should be highly specific and efficient against the target organism in all weather conditions. It should have no effect on the crops to be protected and should not damage ecosystems. In particular, the full complex of beneficial organisms, which support the farmer by keeping down pest populations or maintaining an active soil, should not be disturbed by the control measure. Finally, the applied ingredient has to break down completely into simple, harmless and naturally occurring molecules within a few days and there should be no accumulation of the active ingredient in soil or organisms.

It can be concluded that metaldehyde is a slug control agent that fulfils these prerequisites. Because of its mode of action on the mucus cells of molluscs, it acts in a highly specific and efficient way against slugs and snails in aquatic and terrestrial systems. None of the beneficial organisms investigated showed adverse effects and no phytotoxic effects were observed. Metaldehyde breaks down into acetaldehyde which is a widespread naturally occurring molecule that subsequently gets degraded by micro-organisms into CO_2 and H_2O.

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