**Title: The effects of metaldehyde on non-target aquatic macroinvertebrates:
integrating field and laboratory-based evidence**

Authors

Kieran J. Gething^{1,2*}, Alex Pickwell³, Richard P. Chadd³ and Paul J. Wood¹.

¹Geography and Environment, Centre for Hydrological and Ecosystem Science,
Loughborough University, Loughborough, Leicestershire LE11 3TU, UK.

²School of Science and Technology, Nottingham Trent University, Nottingham, NG11
8NS, UK.

³Environment Agency of England, Lincolnshire & Northamptonshire Area, Stepping
Stone Walk, Winfrey Avenue, Spalding, Lincolnshire, PE11 1DA, UK.

Corresponding author

*Kieran J. Gething - kieran.gething2015@my.ntu.ac.uk

Abstract:

The use of pesticides has historically helped improve agricultural productivity, although their continued use may have unforeseen effects upon the natural environment when not applied appropriately. Metaldehyde is a commercial pesticide widely used to reduce crop losses resulting from terrestrial mollusc damage. However, following precipitation and runoff it frequently enters waterbodies with largely unknown consequences for aquatic fauna. This study represents one of the first attempts to examine its potential effects on aquatic macroinvertebrate communities at sites known to have experienced elevated metaldehyde concentrations alongside unaffected control sites. In addition, a series of laboratory exposures specifically examined the effects of metaldehyde on the survivorship of non-target aquatic mollusc species. When the entire aquatic macroinvertebrate community and aquatic mollusc community were considered, limited differences were observed between metaldehyde affected and control sites based on field data. Laboratory exposures highlighted that for the molluscs examined, gastropods (*Bithynia tentaculata*, *Planorbis planorbis*, *Radix balthica* and *Potamopyrgus antipodarum*) had a greater tolerance to metaldehyde than bivalves (*Sphaerium corneum* and *Corbicula fluminea*). However, the concentrations required to reduce survivorship of all species were much greater than those ever recorded historically under field conditions. The results suggest that the differences in the community composition recorded between sites exposed to elevated metaldehyde concentrations and control sites were probably due to nutrient loading (N and P from agricultural fertilizers) rather than metaldehyde. However, these results do not negate wider concerns regarding metaldehyde use, particularly issues caused when ingested by vertebrate wildlife, livestock or children and pets in domestic settings.

Capsule:

Metaldehyde, a pesticide, frequently enters agricultural water bodies in excess of safe drinking water levels but has limited effects upon non-target invertebrate organisms.

Keywords: Molluscicide, invertebrate, aquatic mollusc, field study, laboratory exposure.

Introduction

Pesticides are a valuable tool in modern agriculture that have historically helped improve productivity (Handford et al. 2015). However, in some instances their use has raised concerns for human health, food and drinking water resource safety (World Health Organization and United Nations Environment Programme, 1990; Nicolopoulou-Stamati et al., 2016; Deng et al., 2019) and more widely in terms of unintended environmental effects (van der Werf, 1996; Carvalho, 2017; Wood and Goulson, 2017). The use of pesticides is heavily regulated in some regions (Karabelas et al. 2009; Handford et al. 2015), such as Europe where the European Union Water Framework Directive 2000/60/EC mandates a maximum permissible concentration of pesticides in drinking water of 0.1 µg/L for a single pesticide (Dolan et al. 2013).

The agricultural sector has benefitted from the use of modern agrochemicals and the availability of plentiful freshwater resources for irrigation, which have collectively helped increased crop yields and minimised damage by pests (FAO 2004; Stewart and Roberts 2012; DEFRA 2013; Handford et al. 2015). However, these practices potentially threaten aquatic biodiversity by reducing water availability, changing the sediment regime and nutrient dynamics, and via the poorly managed use of pesticides (Dudgeon, 2010; Reid et al., 2019; Jiao et al., 2020). Given that biodiversity is a key measure of aquatic ecosystem health (Natural England 2008; Environment Agency 2018), and an important component of freshwater ecosystem service provision (MEA 2005; DEFRA 2011; Parker and Oates 2016; IPBES 2019; UNEP: TEEB 2019), there is an ongoing need to monitor and understand any effects of pesticide use on non-target organisms.

One of the most widely utilised pesticides in the arable agricultural sector is the molluscicide metaldehyde (IUPAC: 2,4,6,8-tetramethyl-1,3,5,7-tetroxocane: Castle et al., 2017). Metaldehyde has been used for over a century and is known to be highly effective against target terrestrial gastropods (slugs and snails), but there have been limited documented effects upon other terrestrial invertebrates (e.g. *Lumbricus terrestris* (Linnaeus 1758): Langan and Shaw 2006; Edwards et al. 2009). In aquatic environments, EC₅₀₋₆₀ of 100->200 mg/L have been reported for the gastropods *L. stagnalis* (Linnaeus 1758), *Planorbarius corneus* (Linnaeus 1758) and the bivalve *Dreissena polymorpha* (Pallas 1771) (Putchakayala and Ram, 2000; European Food

Safety Authority, 2010; Hallett et al., 2016). However, the tolerances of other aquatic taxa and the effects of metaldehyde upon the wider aquatic ecosystem have not been widely quantified.

Metaldehyde use is permitted throughout the European Union, however, during 2018 the UK Department for Environment, Food and Rural Affairs (DEFRA) announced plans to ban metaldehyde from Spring 2020, owing to the “*unacceptable risks [posed...] to birds and mammals*” (DEFRA, 2018). This proposal was subsequently overturned in the UK courts due to lack of evidence regarding metaldehyde’s impact upon non-target organisms. Real world studies of metaldehyde pollution have almost exclusively focused on water security concerns (e.g. Kay and Grayson 2014), with most biological studies being undertaken in the laboratory. As a result, limited field studies examining the biological effects of metaldehyde have been undertaken, making it difficult to assess metaldehyde’s legacy at the landscape scale. Given that freshwater aquatic molluscs (largely gastropods and bivalves) are evolutionarily and physiologically similar to their terrestrial counterparts (Saleuddin and Wilbur 1983; Thorp and Covich 2009; Moreau et al. 2015; Pyron and Brown 2015) it has been hypothesised that these faunal groups may be disproportionately affected by metaldehyde pollution (e.g. Calumpang et al. 1995; Horgan et al. 2014). As a result, the effect of metaldehyde concentrations recorded in surface freshwater bodies upon aquatic molluscs and the frequency at which these concentrations may be reached needs to be established. Therefore, this study examined a six-year ecological monitoring data set from the east of England (UK) alongside laboratory exposure studies to determine the effects of metaldehyde upon macroinvertebrate communities and specific non-target gastropod and bivalve species.

Methods

Data collection

Field Observations

10 sites in the east of England (53.061025°N, 0.195007°W) were selected for detailed investigation in the county of Lincolnshire (Figure S1.). The area has a predominantly arable land use and is serviced by a dense drainage network. Peaks of metaldehyde that exceeded drinking water guidelines ($\geq 0.1 \mu\text{g/L}$) were frequently detected between 2012 and 2018 at five locations (D1-D5), with five control sites where metaldehyde had

not exceeded drinking water guidelines in the historical record, but with comparable physical characteristics also being identified (C1-C5) to provide direct comparisons during analysis. The five metaldehyde-affected sites identified were among those monitored by the Environment Agency (the regulatory body for England) during 2014, the year in which 81% of pesticide pollution events in England involved metaldehyde (Pesticides Forum 2016). During 2015, 100% of serious pesticide-related pollution incidents in England involved metaldehyde, 60% of which occurred within the study area (upstream of D4 and D5) (Pesticides Forum 2017).

A total of 114 aquatic macroinvertebrate samples (60 from D1-5, 54 from C1-5), collected by the Environment Agency via the kick sampling methodology (see Murray-Bligh, 1999) between 2012 and 2018, were available for investigation. Invertebrates were identified to species level, except Diptera larvae which were primarily identified to family level and Ostracoda, Cladocera, Hydracarina and Oligochaeta which were identified as such. Water temperature (°C), pH, conductivity (µS/cm), dissolved oxygen (DO) (mg/L, %), chloride (mg/L), nitrate (µg/L), orthophosphate (mg/L), phosphorus (mg/L) and metaldehyde (µg/L) data, matched to corresponding date and locations of macroinvertebrate samples, were made available for detailed analysis by the Environment Agency and Anglian Water Plc.

Laboratory exposures

In order to advance understanding of the effects on specific non-target organisms, a series of laboratory exposures were also undertaken to test mollusc survivorship rates upon exposure to metaldehyde under controlled conditions. Six taxa were used in the controlled laboratory exposures: four native species i) *Bithynia tentaculata* (Linnaeus, 1758), ii) *Planorbis planorbis* (Linnaeus, 1758), iii) *Radix balthica* (Linnaeus, 1758) and iv) *Sphaerium corneum* (Linnaeus, 1758) and two non-native species i) *Potamopyrgus antipodarum* (Grey, 1843) and ii) *Corbicula fluminea* (Müller, 1774) that are widely distributed. Organisms were collected from waterbodies around Loughborough, UK (51.395918°N, 0.734332°W) using a standard kick net (mesh size 1mm) prior to the commencement of an experimental cycle. All organisms were held in aquaria for approximately 12 h prior to use in the experiment to confirm all individuals were alive and healthy. Exposure tanks were set up and run for 24 h prior to the introduction of organisms to allow the stabilisation of internal conditions.

Each exposure tank contained either 15.0 l of dechlorinated water (control) or 15.0 l of dechlorinated water and a known concentration of metaldehyde (Doff® Slug Killer: 1.5% metaldehyde – all concentrations used were calculated based on reported active ingredient per-unit mass of pellets provided by the manufacturer). Each exposure tank was fitted with an aerator to supply oxygen to the organisms within. Temperature was held at ambient conditions (18-22 °C) within the River Science Laboratory at Loughborough University. At the start of the experiment 20 adult individuals from one of the six tested species were introduced into each exposure tank. All experiments had a 24-hr duration given that metaldehyde peaks ≥ 0.1 $\mu\text{g/L}$ did not typically exceed this duration in the field data series and reflected typical environmental exposure conditions. On completion of the experimental period, organisms were transferred into a clean aquarium and directly observed over a 3-hr period to determine survivorship rates. Each of the six species was tested at four metaldehyde concentrations, with three replicates of each concentration providing a total of 72 individual experiments. All organisms were first tested at 100 mg/L, reflecting EC_{50-60} concentrations recorded by Putchakayala and Ram (2000), the European Food Safety Authority (2010) and Hallett et al. (2016). If survivorship at 100 mg/L was $>80\%$, the four test concentrations were 0, 100, 200 and 300 mg/L (*B. tentaculata*, *P. planorbis* and *P. antipodarum*). If survivorship at 100 mg/L was $<50\%$, the four test concentrations were 0, 25, 50 and 100 mg/L (*C. fluminea*). One species (*S. corneum*) had a mean survivorship of 58% at 100 mg/L so was tested at 0, 50, 100 and 200 mg/L. *R. balthica*, demonstrated a marked decline in survivorship between 100-200 mg/L, and was therefore tested at 0, 100, 150 and 200 mg/L.

Data analysis

Unless otherwise stated, R Studio v.3.5.1 (R-Studio Team 2016) was used to perform statistical analyses and visualise plots. All faunal and environmental matrices were square root transformed in order to satisfy the assumptions of further statistical analyses and facilitate the use of Vegan package in R language for environment and statistical computing v.2.5.3 (Oksanen et al. 2018; R Core Team 2018).

Field Observations

Spring and autumn macroinvertebrate samples from 2012-2018 with corresponding chemical data were extracted for ordination in Canoco v.5.0 (ter Braak and Šmilauer 2012). Data was plotted in two groups, the first containing the entire faunal community. The second group considered only aquatic molluscs reflecting the fact that there may be more pronounced effects upon this section of the community. Given that both the entire faunal community - all taxa (0.1) and the mollusc community (1.1) displayed linear distributions, Redundancy Analysis (RDA) was conducted with a Monte Carlo random permutations test (n=999) to determine the statistical significance of environmental (water chemistry) variables (Lepš and Šmilauer 2003).

To quantify heterogeneity between metaldehyde detected and control sites for the period of known metaldehyde pollution (2012-2018), analysis of similarity (ANOSIM - utilising the Bray-Curtis dissimilarity metric) was conducted on the entire faunal community (114 samples), and a subset containing only aquatic mollusc taxa (114 samples). Subsequently, similarity percentages analysis (SIMPER) was undertaken on the two ANOSIM groups (all fauna and aquatic molluscs) to determine the species responsible for any observed heterogeneity.

Laboratory exposures

For laboratory experiments, mean survival rate across the three replicates for a given concentration were calculated and plotted for each species.

Results

Field data

A total of 52,286 individuals were recorded from all samples. The relative abundance and number of taxa was lower at control sites (C1-5; mean 415 individuals; 201 taxa) compared to metaldehyde detected sites (D1-5; mean 466 individuals; 245 taxa). The most abundant taxon at sites C1-5 was *Asellus aquaticus* (Linnaeus 1758), followed by *Crangonyx pseudogracilis* (Bousfield 1958), Oligochaeta and Hydracarina. The most abundant taxon at sites D1-5 was also *A. aquaticus* followed by *P. antipodarum*, *B. tentaculata*, *C. pseudogracilis*, *Pisidium nitidum* (Jenyns, 1832), Oligochaeta, Cladocera and Chironomidae. Molluscs comprised 18.9% and 19.7% of the taxa recorded at control and metaldehyde detected sites and 27% and 32% of the relative abundance respectively. *Pisidium subtruncatum* (Malm, 1855) (801), *Anisus vortex*

(Linnaeus 1758) (789) and *B. tentaculata* (661) were the most frequently recorded mollusc taxa at control sites with *P. antipodarum* (1,856), *Pisidium nitidum* (1,080) and *B. tentaculata* (1,062) being the most frequently occurring mollusc taxa at sites where metaldehyde had been detected.

Redundancy Analysis (RDA) indicated that water chemistry data from samples collected at metaldehyde detected and control sites accounted for 16.8% of the total variance in the faunal data. Orthophosphate was identified as the most significant variable influencing the community composition (**Table 1a.**). Metaldehyde, accounted for only 2.1% of the community variation and was strongly correlated with Axis 2 along with orthophosphate and phosphorus (**Figure 1.**). Cumulatively these three variables accounted for 6.7% of the variance in the faunal community data. Metaldehyde, orthophosphate and phosphorus were negatively correlated with DO, temperature and pH. Samples from sites where metaldehyde had been detected (particularly D2 and D4) predominately plotted in the upper quadrants of the ordination. In contrast, samples from control samples typically plotted on the negative end of Axis 2. However, ANOSIM indicated a high degree of similarity among the assemblages recorded at control and metaldehyde detected sites (ANOSIM $R= 0.265$; $P= 0.001$). SIMPER highlighted that 30.7% of the dissimilarity observed was attributable to 10 taxa, three of which were molluscs (*P. nitidum*, *P. antipodarum* and *Valvata piscinalis* (Müller, 1774)) (**Table 2a.**). Hydracarina was the most significant taxon driving dissimilarity between control and metaldehyde detected sites (4.1%) followed by *A. aquaticus* (3.8%).

The 10 environmental variables were able to account for 21.6% of the total variance in the community composition of mollusc communities from control and metaldehyde detected sites. Orthophosphate accounted for the greatest amount of variance (5.0% - **Table 1b.**); being strongly associated with both phosphorus and metaldehyde concentrations (**Figure 2.**). Metaldehyde was the least significant variable accounting for only 1.2% of the variance in the mollusc data. Most control site samples (with the exception of samples from C3) cluster together and were characterised by higher DO, pH, temperature and conductivity. Many samples from sites D2 and D4 were characterised by higher metaldehyde concentrations. A significant ANOSIM, indicated a higher degree of heterogeneity between metaldehyde detected and control samples

(ANOSIM $R= 0.467$; $P= 0.001$) for the mollusc community compared to the equivalent analysis for the entire faunal community. SIMPER attributed 60.1% of the dissimilarity to 10 taxa (**Table 2b.**), the most influential of being *P. nitidum* (9.4%), *P. antipodarum* (9.1%) and *V. piscinalis* (7.5%). All the mollusc taxa identified by SIMPER, with the exception of *Physa fontinalis* (Linnaeus 1758), were more common at metaldehyde detected sites. Both gastropods and bivalves were identified in the SIMPER, indicating both were significantly associated with differences between communities from control and metaldehyde detected sites.

Laboratory exposures

Gastropods displayed higher survivorship than bivalves when exposed to metaldehyde under controlled laboratory conditions. Operculate gastropods consistently displayed a higher mean survivorship than pulmonates at metaldehyde concentrations ≤ 200 mg/L; above this threshold results were more variable. The mean survivorship of *C. fluminea* declined rapidly with only a 7% survivorship at metaldehyde concentrations of 100 mg/L (**Figure 3a.**), while *S. corneum* had a survivorship of 3% at 200 mg/L (**Figure 3b.**). The most rapid decline in survivorship for both *C. fluminea* and *S. corneum* occurred at concentrations >50 mg/L. The operculate gastropod *P. antipodarum* had a 100% survivorship at metaldehyde concentrations ≤ 200 mg/L, but this declined rapidly to a 35% survivorship at 300 mg/L (**Figure 3c.**). *B. tentaculata* displayed very little reduction in survivorship at increased metaldehyde concentrations (**Figure 3d.**) with $>90\%$ survivorship at metaldehyde concentrations of 300 mg/L. Whilst *P. planorbis* displayed a similar response to that of *B. tentaculata* at metaldehyde concentrations up to 100 mg/L, the species had lower mean survival rates at concentrations >100 mg/L (**Figure 3e.**). *R. balthica* displayed the most marked response and lowest survivorship of the gastropods studied at metaldehyde concentrations >100 mg/L (**Figure 3f.**).

Discussion

Metaldehyde poses a potential threat to water security and terrestrial vertebrates (Lapworth et al., 2012; Castle et al., 2017; Pesticides Forum, 2017, 2018), however, its potential impact upon aquatic communities has remained largely unexplored until now. Concentrations of metaldehyde in European rivers have exceeded WFD limits for safe drinking water over the past decade (Lazartigues et al., 2012; Kay and Grayson,

2014), although research on the response of aquatic fauna remained rooted in laboratory studies. This study therefore sought to utilise field data from sites known to exceed metaldehyde drinking water standards to examine the legacy of metaldehyde in agricultural catchments upon non-target aquatic macroinvertebrate communities and specifically mollusc species. This research is particularly timely given the recent reversal of the ban on metaldehyde use across the agricultural sector in the UK (Water UK, 2019).

Aquatic macrofaunal community composition at metaldehyde detected and control sites

Differences in macroinvertebrate communities from control sites and sites where elevated metaldehyde had been recorded were marginal, but statistically significant. Control sites were more homogenous than sites subject to elevated metaldehyde concentrations. Three species were recorded in higher abundances at sites subject to elevated metaldehyde concentrations (*A. aquaticus*, *C. pseudogracilis* and *P. antipodarum*), and are noted for their association with nutrient enrichment (Dick et al., 1999, 1998; Simčič and Brancelj, 2006; Alonso and Castro-Díez, 2008). Detailed examination of the mollusc community indicated even larger differences between control sites and those sites subject to greater metaldehyde concentrations than for the entire macroinvertebrate community. Analysis of environmental (water chemistry) variables using the Monte Carlo random permutations test, for both the entire faunal community and the mollusc community, indicated that metaldehyde did not appear to have a significant effect. This analysis indicated that, rather than metaldehyde, phosphate and orthophosphate (probably originating from agricultural fertilisers), and differences in pH and DO were the primary drivers of community composition; with the effects being more pronounced on the mollusc community.

Aquatic molluscs have previously been shown to display limited response to pesticide pollution events (Raven and George, 1989). The maximum metaldehyde concentration recorded during this study (4.4 µg/L) was 22,523 times lower than the >100 mg/L concentration at which lethal effects have previously been observed (e.g. Hallett et al. 2016). Therefore, it is likely that the impact of a single metaldehyde pollution event upon the aquatic mollusc community was limited. Increased duration of molluscicide exposure can increase mollusc mortality (Doherty and Cherry, 1988), however, the

duration of metaldehyde peaks $\geq 0.1 \mu\text{g/L}$ recorded in the field were short (c. 24 h). Operculate and bivalve taxa have been shown to seal their shells to withstand short term exposure to pesticides (Wallace 1990; Gosling 2004; Pandey and Kulkarni 2007). In contrast, pulmonates do not have an operculum and, therefore, may be at greater risk of mortality, even during short-term pollution events. It is however likely that some, if not most, pulmonate taxa possess the ability to withstand high concentrations of metaldehyde over an extended period, with Hallett et al. (2016) noting the ability of *L. stagnalis* to internally detoxify metaldehyde.

Acute impacts of terrestrial molluscicide pollution upon aquatic mollusc species under laboratory conditions

To date, few authors have attempted to quantify the effect of metaldehyde upon non-target aquatic mollusc species. Increasing metaldehyde concentrations reduced the survivorship of all six species examined, although the effect was highly variable. There are three factors most likely to be responsible for the variability observed, reflecting species differences in: (1) feeding mode, (2) respiratory mode, and (3) metaldehyde excretory efficiency.

Feeding mode is potentially of significance, as ingestion has previously been noted to affect the behaviour of aquatic molluscs (Egeler et al. 2007; European Food Safety Authority 2015). Mills et al. (1992) reported that ingestion of metaldehyde resulted in a reduction in feeding and thus the rate at which metaldehyde could be consumed prior to a lethal concentration being reached within an organism. The four gastropod species examined feed via a radula (an arrangement of small teeth located in the mouth: Mendel and Bradley 1905; Saleuddin and Wilbur 1983). This is in contrast to the bivalve taxa, both of which feed predominately via filtration (Thorp and Covich 2009; Pyron and Brown 2015). *S. corneum* and *C. fluminea* displayed lower survivorship rates at lower metaldehyde concentrations. Filter-feeding may have resulted in large volumes of metaldehyde contaminated water passing directly through the organism, thus potentially elevating the effects at lower concentrations. As filtration rates are noted to vary as a function of size in many bivalves (Wagner 1976; Riisgård 2001; Sylvester et al. 2005), it is likely that larger volumes of metaldehyde entered larger individuals more quickly and increased mortality. Further investigation would be required to determine if there is a relationship between body size, filtration and the

effects of metaldehyde. The two gastropod species observed to directly ingest metaldehyde pellets (*P. planorbis* and *R. balthica*) demonstrated significant reductions in survivorship at concentrations ≤ 200 mg/L suggesting that ingestion may be an important factor in determining mollusc survivorship.

Differences in mode of respiration may also help explain the difference in response of the taxa examined. Across the six species examined, two modes of respiration are utilised. First, gills allowing gaseous exchange over an internal membrane to obtain oxygen, are utilised by *S. corneum*, *C. fluminea*, *B. tentaculata* and *P. antipodarum* (Thorp and Covich 2009). Pulmonates (*P. planorbis* and *R. balthica*), also utilise gaseous exchange across membranes underwater via highly vascularised tentacles but can also breathe atmospheric air via a primitive lung when aquatic conditions are unfavourable (Pyron and Brown 2015). However, no difference in survivorship / tolerance was observed in the laboratory exposures and no pulmonate individual attempted to emerge from the water after metaldehyde was added to the exposure tank. This is likely to be because cues which prompt an escape response relate more closely to oxygen availability than a response to a potential biocide.

In addition to the ingestion of metaldehyde, the rate of excretion is likely to influence survivorship. Studies examining the ability of molluscs to excrete metaldehyde are limited. Research on the pulmonate gastropod *L. stagnalis* suggested that it was able to internally detoxify metaldehyde with no observable effect up to 36 mg/L (Hallett et al. 2016); although the mechanism by which detoxification and excretion occurred was not specified. The results of the current investigation demonstrate similarly high survivorship at 36 mg/L, particularly for gastropod taxa with the most marked reductions occurring at concentrations over 100 mg/L for gastropod species. The concentration was an order of magnitude higher than those reported for UK rivers (<10.0 $\mu\text{g/L}$: Kay and Grayson 2014). Therefore, the results of the laboratory exposures clearly demonstrate that much higher concentrations of metaldehyde than the maximum observed in the field (4.4 $\mu\text{g/L}$) would be required to reduce the survivorship of molluscs.

Conclusion

The results of field-based research at sites where the greatest concentrations of metaldehyde have been recorded indicate that its effects on non-target aquatic macroinvertebrates within an agricultural setting were so minor that they could not be detected. Although metaldehyde concentrations exceeded drinking water guidelines on multiple occasions at sites examined during the study period, these concentrations were orders of magnitude lower than those documented to influence aquatic faunal communities and individual aquatic mollusc species. The results recorded suggest that the differences observed between metaldehyde detected and control sites probably reflect differences in nutrient concentrations (orthophosphate) resulting from land use practices associated with specific crops (e.g., brassica where agricultural fertilisers and metaldehyde may be used more frequently). Laboratory exposures examining individual mollusc species highlighted a wide range of tolerances. These findings also highlight that real-world metaldehyde concentrations and exposure times recorded in UK rivers were almost certainly lower than those required to have a lethal effect on non-target aquatic macroinvertebrates. The overall results of this research drawing on field based ecological monitoring and laboratory exposures highlight that metaldehyde poses a limited threat to non-target aquatic faunal communities based on current levels of exposure. However, it should be stressed that these results do not negate wider concerns regarding metaldehyde use, particularly issues caused when ingested by vertebrate wildlife, agricultural livestock and domestic pets or the genuine public health concern when accidentally ingested by children when the pesticide is used in a domestic setting.

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Figure captions

Figure 1. RDA biplot of chemical variables and community samples from control (black) and metaldehyde detected (white) sites.

Figure 2. RDA biplot of chemical variables and mollusc community samples from control (black) and metaldehyde detected (white) sites.

Figure 3. Mean survival rate of (A) *Corbicula fluminea*, (B) *Sphaerium corneum*, (C) *Potamopyrgus antipodarum*, (D) *Bithynia tentaculata*, (E) *Planorbis planorbis* and (F) *Radix balthica* upon exposure to differing metaldehyde concentrations.

Figure S1. Study sites within Lincolnshire, North-Cambridgeshire and Rutland, UK.

Table 1. Monte Carlo Permutation results of chemical variables impacting (A) all taxa and (B) mollusc taxa recorded 2012-18.

A. Entire faunal community				B. Mollusc community			
Name	Variation explained (%)	pseudo-F	P	Name	Variation explained (%)	pseudo-F	P
Orthophosphate	3.3	2.1	0.016	Orthophosphate	5.0	3.3	0.004
Chloride	3.3	2.2	0.020	Chloride	3.5	2.3	0.010
Conductivity	2.6	1.8	0.040	Phosphorus	3.1	2.1	0.026
Metaldehyde	2.1	1.4	0.166	pH	2.7	1.9	0.044
Temperature	2.4	1.6	0.078	Nitrate	2.0	1.4	0.152
DO (%)	1.6	1.1	0.328	Temperature	1.7	1.2	0.270
DO (mg/L)	2.2	1.5	0.104	DO (mg/L)	1.5	1.0	0.372
Nitrate	2.1	1.5	0.088	DO (%)	3.8	2.8	0.008
pH	1.1	0.8	0.600	Conductivity	1.6	1.2	0.300
Phosphorus	1.3	0.9	0.574	Metaldehyde	1.2	0.8	0.556

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693 **Table 2.** Taxa responsible for the largest proportion of dissimilarity (SIMPER) between
694 metaldehyde detected and control sites for the (A) entire faunal community, and (B)
695 the mollusc community.

A. Entire faunal community		B. Mollusc community	
Taxa	Cumulative dissimilarity accounted for (%)	Taxa	Cumulative dissimilarity accounted for (%)
<i>Hydracarina</i>	4.1	<i>Pisidium nitidum</i>	9.4
<i>Asellus aquaticus</i>	7.9	<i>Potamopyrgus antipodarum</i>	18.5
<i>Oligochaeta</i>	11.7	<i>Valvata piscinalis</i>	26.0
<i>Chironomidae</i>	14.8	<i>Bithynia tentaculata</i>	32.5
<i>Ostracoda</i>	17.9	<i>Anisus vortex</i>	38.8
<i>Crangonyx pseudogracilis</i>	20.8	<i>Physa fontinalis</i>	44.9
<i>Pisidium nitidum</i>	23.7	<i>Radix balthica</i>	49.7
<i>Potamopyrgus antipodarum</i>	26.4	<i>Theodoxus fluviatilis</i>	54.0
<i>Valvata piscinalis</i>	28.6	<i>Sphaerium comeum</i>	57.9
<i>Sigara dorsalis</i>	30.7	<i>Sphaerium rivicola</i>	60.1

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Figure 1.

Figure 2.

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Figure 3.

Figure S1.