Wyre Rivers Trust

# Diazinon

Upper River Calder

# Contents



# List of abbreviations

| | | | |
|---|---|---|---|
| AA | annual average | AChE | acetylcholine esterase |
| AF | assessment factor | ANOVA | analysis of variance |
| ASTM | American Society for Testing and Materials | BCF | bioconcentration factor |
| Bw | body weight | CAS | Chemical Abstracts Service |
| CL | confidence interval | DT50 | time taken to degrade by 50% |
| EC50 | concentration effective against 50% of the organisms tested | ECB | European Chemicals Bureau |
| EQS | Environmental Quality Standard | FPD | flame photometric detection |
| GC | gas chromatography | GC-MS | gas chromatography/mass spectrometry |
| GLP | Good Laboratory Practice (OECD) | GM | geometric mean |
| LC50 | concentration lethal to 50% of the organisms tested | LOAEL | lowest observed adverse effect level |
| LOD | limit of detection | LOEC | lowest observed effect concentration |
| lt | long term | MAC | maximum allowable concentration |
| MATC | maximum allowable toxicant concentration | NA | not applicable |
| ND | no data | NOAEL | no observed adverse effect level |
| NOEC | no observed effect concentration | NPD | nitrogen phosphorus detection |
| NR | not reported | OECD | Organisation for Economic Co-operation and Development |
| PNEC | predicted no-effect concentration | | |

# Introduction

Diazinon is an organophosphate (OP) insecticide and acaricide (a chemical which kills mites & ticks), which acts as a contact, stomach and respiratory poison. In common with other OP's diazinon's toxic action is achieved by inhibiting acetylcholinesterase, an enzyme essential for normal nerve impulse transmission.

It has been used throughout the world to control a wide range of sucking and chewing insects and mites on a wide range of crops including deciduous fruit trees, citrus fruit, bananas, vegetables, potatoes, coffee, cocoa and rice. It's also used to control agricultural soil dwelling insects, and is applied as sheep dip to control ectoparasites such as sheep scab and blow fly strike.

Introduced in the 1950's by GEIGY (later CIBA-GEIGY – NOVARTIS). The world health organisation classifies diazinon as a class II "moderately hazardous" pesticide. Once used abundantly worldwide diazinon was appreciated for the control and prevention of sheep scab due to its affinity for wool lipids.

Usage of diazinon products in sheep dip strongly declined after countries imposed very strict safety precautions and dip wash disposal regulations (e.g. the UK and Australia) for all dips in the late 1990's. Soon after NOVARTIS, the market leader for diazinon products for sheep, divested all its OP's for strategic reasons. Today in many countries sprays and dips containing diazinon or other OP's have been vastly replaced by injectable macrocyclic lactones (Doramectin, Ivermectin, and Moxidectrin) that have become affordable, are much more convenient than sprays & dips and as effective. Diazinon is still abundantly used worldwide in insecticide-impregnated ear-tags for fly control on cattle and flea collars for pets (Mole, 2010).

# Diazinon as an agricultural pesticide, biocide or a veterinary medicine within the EU

Active substances used in plant protection products are currently approved for use at EU level by inclusion in Annex I to Council Directive 91/414/EEC (Mole, 2010). EU approval for plant protection products containing diazinon was withdrawn on 6$^{th}$ December 2007 with the last possible legal use to expire on 6$^{th}$ December 2008. Approval was withdrawn because; the available data did not demonstrate that worker, operator or bystander exposure levels were acceptable and there was insufficient information on some highly toxic impurities.

When used as a biocide, diazinon had to be approved for use within the EU by inclusion in Annex 1, 1a or 1b to Directive 98/8/EC, the Biological Products Directive. However on the 8$^{th}$ February 2010 the European Commission withdrew the approval for the use of diazinon in biocidal products. This was due to the non-submission of the required information dossier rather than any identified concern. Diazinon was not permitted to be used within the EU after 1$^{st}$ March 2011.

In the US the Environmental Protection Agency (EPA) phased out all residential uses of diazinon in 2004 to mitigate the risk to the general population, especially children, as it was believed that there was a risk of significant acute toxicity to humans.

Diazinon is also approved for use at EU level as a veterinary medical product in accordance with EU Directive 2001/82/EC. This allows diazinon to be used in domestic cat and dog flea collars as well as for sheep dips. Currently there are two UK approved sheep dips containing diazinon (Paracide 62 & Osmonds Golden Fleece Sheep Dips) (Health, 2017) and a number of products for controlling fleas and ticks on cats and dogs, all of which are collars.

There are clearly three distinct regulatory regimes in the EU all concerned with the same active ingredient. One of these has identified issues with the toxicity of diazinon and withdrawn its approval for use. The EPA in the US has also withdrawn the use of diazinon in residential settings due to its potential toxic effects on people and the environment.

Yet it is allowed in sheep dip and flea collars within the UK. Regulation, whilst affording protection towards humans from the effect of diazinon, by allowing the use of sheep dip and flea collars with the active ingredient diazinon, leaves farmers and pet owners exposed.

# Diazinon in the environment

Sheep dipping can play an important part in the maintenance of good animal welfare. The treatment chemicals used are effective against parasites, but can also be hazardous to the aquatic environment if not used carefully. Using best practice and careful planning can help to reduce the amount of chemicals used, thus lowering farmer costs and reducing the risks to groundwater. Groundwater and surface waters are interlinked and therefore pollution of one can seriously affect the quality of the other. Groundwater is an important source of drinking water in rural areas and is essential to the maintenance of wetlands and the wider aquatic environment. Groundwater moves into watercourses and helps to maintain river flow in periods of dry weather.

Diazinon applied to soils can last for weeks or even months depending on the soil environment. Diazinon has the potential to dissolve in water, move through soils and contaminate groundwater. It was commonly found in drinking water sources before the phase-out of residential uses in 2004. Since the phase-out, diazinon has been detected in drinking water sources much less often.

Bacteria, sunlight and other chemical reactions break down diazinon in the environment over time into other chemicals. After diazinon has been applied, some of the diazinon can escape into the surrounding air, a process called volatilization. Diazinon can be taken up by plants and moved throughout. The amount of diazinon in or on plants will decrease over time as the diazinon is broken down by the plant and by the environment.

Diazinon has a low persistence in soil. The half-life is 2 – 4 weeks (Bennet, 2013). Bacterial enzymes can speed the breakdown and have been used in treating emergency situations such as spills. Diazinon seldom migrates below the top half inch in soil, but in some instances, it may contaminate groundwater.

The breakdown rate of Diazinon in water depends on its acidity. At high acidity levels, one half of the compound will disappear in 12 hours while in neutral solution Diazinon will take 6 months to degrade to one half of the original concentration. It is hydrolytically stable with a half-life in natural waters of several days but undergoes microbial degradation. Diazinon is moderately lipophilic (log Kow 3.1–4.0) and so will tend to partition into sediment and biota. Its primary mode of action is through the inhibition of cholinesterases in the nervous system; invertebrates are particularly sensitive (Lepper, et al., 2007).

In plants, a low temperature and a high oil content tend to increase the persistence of Diazinon. Generally, the half-life is rapid in leafy vegetables, forage crops and grasses. The range is from 2 to 14 days. In treated rice plants only 10% of the residue was present after 9 days.

Diazinon is absorbed by plant roots when applied to the soil and translocated to other parts of the plant.

# Toxicity to freshwater organisms

Single species acute toxicity data are available for eight different taxonomic groups, i.e. algae, crustaceans, fish, amphibians, insects, molluscs, annelids, and planarians. Chronic toxicity data are available for algae, crustaceans, fish, insects and rotifers.

Fish, crustaceans and insects are the most sensitive species for both chronic and acute effects of diazinon. However, algae as well as molluscs, planarians and annelid worms appear to be of low sensitivity, whereas amphibians may belong to the more sensitive taxa.

Diagrammatic representations of the available freshwater data (cumulative distribution functions) for diazinon are presented in Figures 1 and 2. These diagrams include all data regardless of quality and provide an overview of the spread of the available data (Lepper, et al., 2007)

**Figure 1**

**Cumulative distribution function of freshwater long-term data (µg l-1) for diazinon**

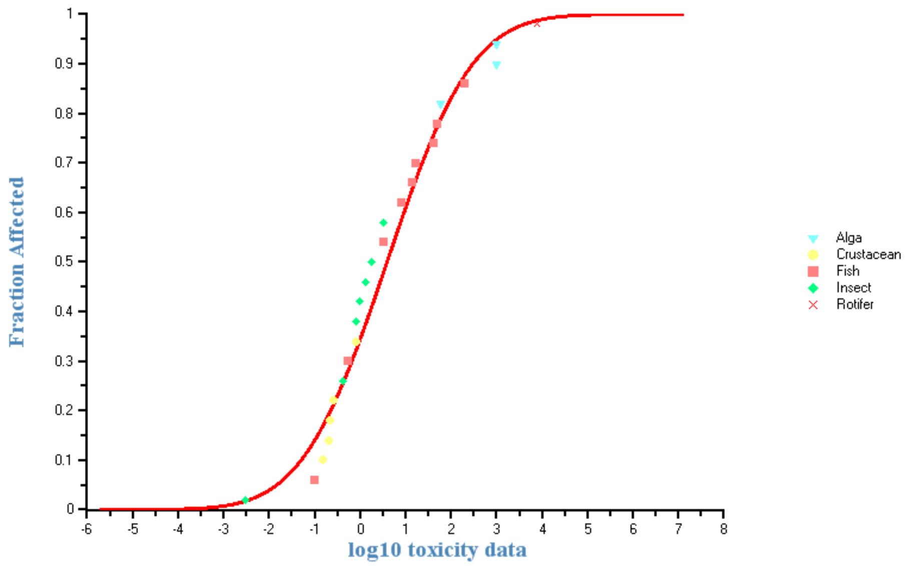
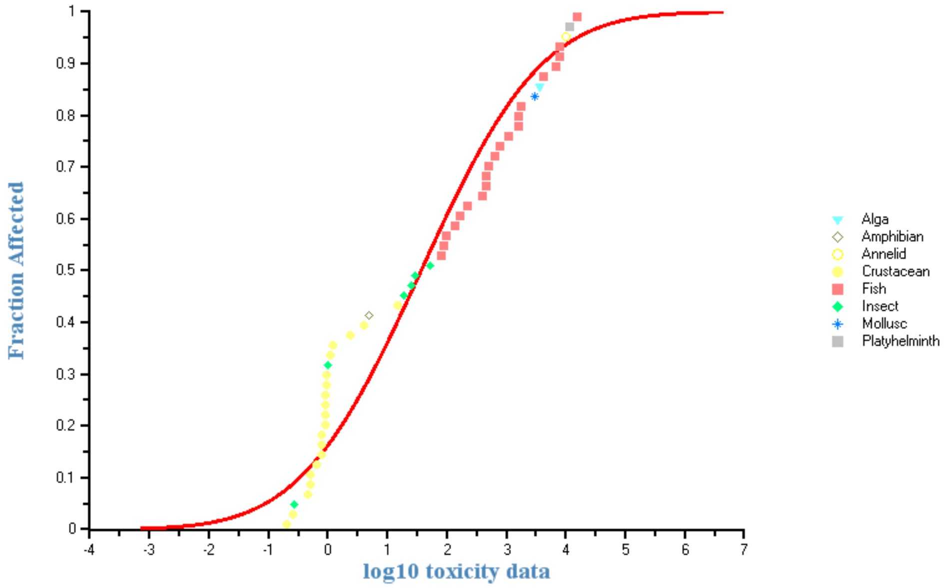


**Figure 2 Cumulative distribution function of freshwater short-term data (µg l-1) for diazinon**




In addition, two outdoor simulated ecosystem studies of a pond and a stream community are available (Table 2.6):

- (Giddings, et al., 1995) performed a microcosm study with 18 fibreglass tanks (3.2 m in diameter and 1.5 m in depth, sediment and water from natural ponds and each tank stocked with 40 juvenile bluegill sunfish). Eight loading rates with two tanks for each level plus two controls were used. The ponds received three consecutive diazinon applications at 7-day intervals, with single applications ranging from 2 to 500 µg l-1 and corresponding time weighted average (TWA) concentrations of 2.4, 4.3, 9.2, 22, 54, 117, 205, and 443 µg l-1 (TWA over 70 days).
- The most sensitive taxa in the mesocosms were chironomid insects of the families Pentaneurini, Ceratopogonidae and Cladocera (daphnids). The latter were significantly reduced at all treatment levels and for the entire post-treatment period of 70 days, i.e. no observed effect concentration (NOEC) <2.4 µg l-1. Effects on various other zooplankton and macroinvertebrate taxa occurred at diazinon concentrations of 9.2 µg l-1 (TWA). Fish biomass was reduced at 22 µg l-1 and survival at 54 µg l-1. Dragonflies, some dipteran insects, and plants were not adversely affected by diazinon at the highest concentration tested (443 µg l-1 TWA).
- (Arthur, et al., 1983) evaluated the effects of diazinon on macroinvertebrates in three outdoor experimental channels. One channel served as a control and two channels as low and high treatments. The low and high treatment channels were continuously dosed at either 0.3 or 3 µg l-1 nominal diazinon concentrations for 12 weeks, which was then increased to 6 and 12 µg l-1 for four weeks, and finally the high treatment was increased to 30 µg l-1 and the low treatment channel returned to ambient. Only the first 12-week dosing regime achieved nominal diazinon levels (0.3 and 3 µg l-1) as indicated by analytical measurements; the latter two dosing regimes did not reach the intended levels.
- No consistent interchannel differences were observed in total macroinvertebrate abundance or in species diversity indices. Hyalella was the most sensitive species, exhibiting substantially higher (3.5–7.8 times) drift rates in the 0.3 µg l-

1 dosed channel relative to the control channel. Hyalella had sharply reduced population levels at diazinon concentrations as low as 5 µg l-1.

- Macroinvertebrate diazinon tolerance from most tolerant to least tolerant was observed as: flatworms, physid snails, isopods and chironomids (most tolerant); leeches and the amphipod Crangonyx (less tolerant); and the amphipod Hyalella, mayflies, caddisflies and damselflies (sensitive).
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# Toxicity to saltwater organisms

Single species test toxicity data for marine organisms are available for five different taxonomic groups, i.e. crustaceans, fish, molluscs (bivalves), annelids and echinoderms (sea urchins). Chronic toxicity data are only available for one crustacean and two fish species. Consequently, acute as well as chronic toxicity data are lacking for algae. Results of higher tier mesocosm or field studies with marine aquatic organisms are also unavailable.

Based on the limited data available, crustaceans appear to be the most sensitive taxonomic group with respect to the acute toxicity of diazinon. However, conclusions about the toxicity of other taxonomic groups cannot be drawn due to a lack of data. Results of the available long-term tests with marine crustaceans and fish are within the range of results obtained for freshwater organisms.

Diagrammatic representations of the available saltwater data (cumulative distribution functions) for diazinon are presented in Figures 3 and 4. These diagrams include all data regardless of quality and provide an overview of the spread of the available data.

**Figure 3: - Cumulative distribution function of saltwater long-term data (µg l-1) for diazinon**

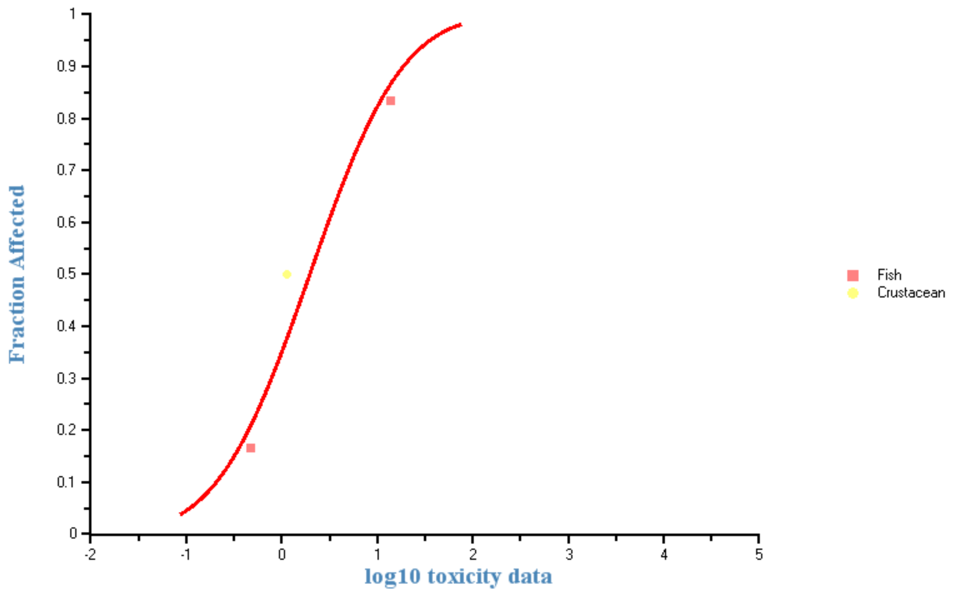
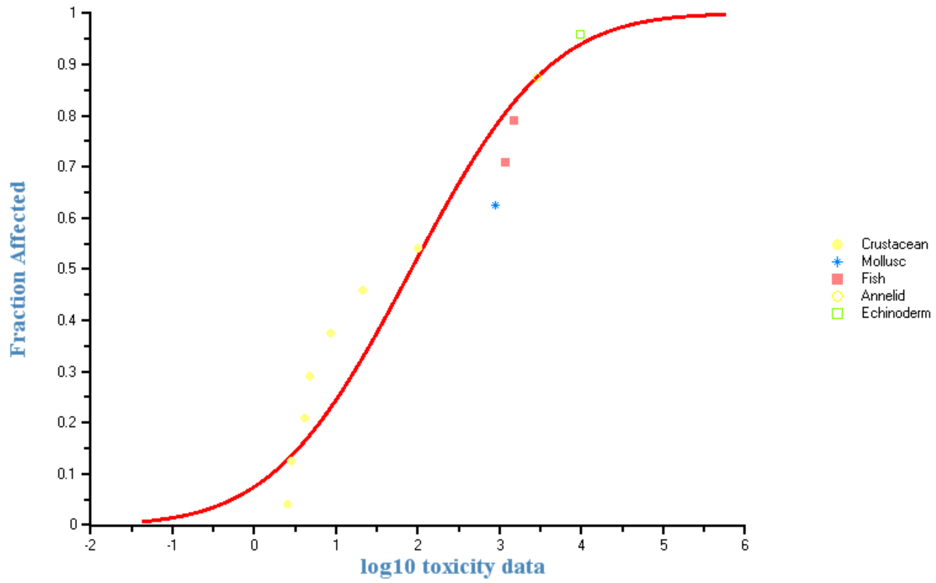


**Figure 4: - Cumulative distribution function of saltwater short-term data (µg l-1) for diazinon**




# Long-term predicted no-effect concentration for freshwaters

Reliable chronic data are available for invertebrates and fish. Recent studies have revealed significant reductions in olfactory responses of male Atlantic salmon (*Salmo salar*) following short-term exposure to 0.3 µg l-1 diazinon, with a no observed effect concentration (NOEC) of 0.1 µg l-1. Although the exposure period was only 30 minutes, effects on reproductive steroid concentrations, the sensitivity of the olfactory epithelium and sperm volumes were observed, with important long-term implications for reproductive success. These data are supported by similar NOECs for reproduction in the crustaceans *Ceriodaphnia dubia, Daphnia magna* and *Gammarus pseudolimnaeus*. The standard assessment factor of 10 applied to the Atlantic salmon NOEC of 0.1 µg l-1 is recommended, resulting in a PNEC freshwater_lt of 0.01 µg l-1.

This is similar to the existing EQS of 0.03 µg l-1 for sheep dip insecticides (the combined concentrations of diazinon, chlorfenvinphos, propetamphos, coumaphos and fenchlorphos) based on a Daphnia magna NOEC of 0.15 µg l-1, to which an assessment factor of 5 was applied.

# Short-term predicted no-effect concentration for freshwaters

Good quality data are available from acute studies with eight taxa including fish, insects and crustaceans. The most sensitive of the insects and crustaceans are at least an order of magnitude more sensitive than the most sensitive fish species. The lowest reliable effects concentration is a 96-hour LC50 of 0.2 µg l-1 to the freshwater shrimp *Gammarus fasciatus*. The specific mode of action of diazinon, coupled with the indications that this species is likely to be among the most sensitive taxa, allows a reduced assessment factor (10) to be applied instead of the default value of 100, resulting in a PNEC freshwater_st of 0.02 µg l-1.

This is five times lower than the existing EQS of 0.1 µg l-1 for sheep dip insecticides (the combined concentrations of diazinon, chlorfenvinphos, propetamphos, coumaphos and fenchlorphos) generated using a smaller assessment factor (2) applied to the same critical data, as permitted by the method used to derive the EQS.

## Long-term predicted no-effect concentration for saltwater's

The limited data suggest similar sensitivities of freshwater and saltwater species, but the greater taxonomic diversity of marine organisms compared with those living in freshwaters introduces greater uncertainty into the prediction of a saltwater PNEC. Nevertheless, in the absence of reliable chronic saltwater toxicity data, a saltwater PNEC may be based on freshwater data. However, an assessment factor of 10 applied to the lowest freshwater chronic NOEC (0.1 µg l-1 for olfactory responses in Atlantic salmon) is considered adequate because evidence from acute tests suggests that long-term NOECs generated for these saltwater taxa would not be lower than those already available. This results in a PNEC saltwater_lt of 0.01 µg l-1, identical to the PNEC freshwater_lt.

This is similar to the existing EQS of 0.03 µg l-1 for sheep dip insecticides (the combined concentrations of diazinon, chlorfenvinphos, propetamphos, coumaphos and fenchlorphos), which was 'read across' from the freshwater long-term value.

## Short-term predicted no-effect concentration for saltwater's

Five taxa are represented among the saltwater acute toxicity dataset, including crustaceans, which are clearly much more sensitive than the other species tested. Acute effect concentrations of 2.5–5.6 µg l-1 were reported in reliable studies with the copepod *Acartia tonsa*, the shrimp *Palaemonetes pugio* and the mysid shrimp *Americamysis bahia*.

Although the Annex V guidance does not specifically address short-term PNECs for the protection of marine species, the general guidance on short-term effects was followed. An assessment factor of 10 applied to the Acartia 96-hour LC50 of 2.57 µg l-1 was recommended, resulting in a PNEC saltwater_st of 0.26 µg l-1. This assessment factor is justified on the basis that, as a crustacean, Acartia tonsa is probably amongst the most sensitive marine species to this insecticide.

This PNEC is slightly higher than the existing EQS of 0.1 µg l-1 for sheep dip insecticides (the combined concentrations of diazinon, chlorfenvinphos, propetamphos, coumaphos and fenchlorphos) based on 'reading across' from the freshwater short-term EQS.

## Predicted no-effect concentration for sediment and secondary poisoning

Although the lipophilicity of diazinon would result in partition from water to sediment, there are insufficient sediment toxicity data to derive a PNEC sediment. For both freshwater and saltwater, PNECs based on the risks of secondary poisoning to mammals and birds (0.06 µg l-1) are higher than those derived for the protection of aquatic life and so do not influence the development of EQSs for diazinon.

# River Wyre

There are numerous environmental impacts arising from farming processes. These impacts include effects to water bodies, humans and economic costs. Due to the nature of the potential contaminants and the common pathways associated with rural settings, such as diffuse pollution, environmental impacts not only affect the immediate vicinity but have the ability to spread to greater distances.

Through a catchment-based approach at a landscape scale, Wyre Rivers Trust will advise and work with local farmers and landowners to help solve the issues surrounding diffuse pollution.

Adopting this approach will lead to the development of a more appropriate River Basin Management Plan, which underpins the Water Framework Directive (WFD). The approach will also provide a platform for engagement and discussion to highlight the main challenges within the local catchment.

Modern-day agricultural practices often require high levels of fertilisers and manure; leading to high nutrient (e.g. nitrogen and phosphorus) surpluses that are transferred to water bodies through various diffuse processes. Excessive nutrient concentrations in water bodies, however, cause adverse effects by promoting eutrophication, with an associated loss of plant and animal species. In high nutrient waters with sufficient sunlight, algal slimes can cover stream beds, plants can choke channels and blooms of plankton can turn the water murky green. Oxygen depletion, the introduction of toxins or other compounds produced by plants, reduced water clarity and fish kills can also result. Excess nutrient levels can be detrimental to human health.

The UK's most common impacts are associated with the use of pesticides, nitrogen compounds, livestock waste and soil erosion. Sheep dips are a prime example of a use of pesticides within the agricultural industry. One area of concern identified in an article by Skinner et al 1997, gave light to the extent to which pesticides reach rivers and lakes by leaching and runoff, since this may lead to impacts on aquatic life, humans and contamination of drinking water.

The potential for Diazinon to enter surface waters in the Wyre catchment comes from its use in sheep dip. Whilst talking with local upland farmers they have confirmed that dipping of sheep is preferred (but not carried out by all farmers in the area) rather than an oral treatment for sheep Scab. This is due in part to oral treatments containing antibiotics which the sheep start to develop a resistance too, causing potential future issues if the flock need other veterinary treatments. And secondly due to the upland environment in which the flock live, oral treatments aren't successfully treating the rate and frequency of which fly strike can happen. (anonymous, 2018).

The effects of Diazinon have been tested on many fish species both salt and fresh water, invertebrates and crustaceans. A sample of these results can be seen in Table 1. Compiled to show the impacts on species relevant to the River Wyre Catchment, affecting all aquatic species throughout the fresh water food chain. For a complete review of Diazinon effects and tolerances see Lepper, et al., 2007.

# Implications for the River Wyre catchment

Whilst Diazinon effects many species within a river system its impact on key stone species such as Atlantic Salmon and Brown Trout can be associated as its greatest impacts. Diazinons effect to migratory fish species ability to migrate and reproduce could and would have a detrimental effect on Salmon and Brown Trout stocks within the River Wyre Catchment.

It is therefore imperative that every effort is made for Diazinon to be inhibited from entering Wyre catchment water courses. As part of this process Wyre Rivers Trust are conducting a study using ChemCatchers to monitor levels of acid herbicides and Diazinon within the Wyre catchment.

Using a method of passive sampling Wyre Rivers Trust are monitoring three sites throughout the middle section of the of the river to determine the levels of both herbicides and Diazinon. With these results we can pin point pollution incidents and trace back to the source of the incident. We can then offer on farm advice and put in place mitigation measures to prevent any further incidents.

Marshaw Wyre at Marshaw.

# Working with local farmers and landowners

Wyre Rivers Trust is committed to working with local farmers, landowners and strategic partners to keep the river Wyre in good ecological condition. By engaging with farmers, we can offer advice on diffuse pollution issues and offer solutions such as fencing off water courses, soil management techniques and bridging ford and stock river crossings etc. Use of pesticides, slurry spreading and sheep dipping.

Farmers involved with sheep dipping operations and purchasing organophosphates (OP) in England, Wales and Northern Ireland must be properly trained (have a Certificate of

Competence in Safe Use of Sheep Dips) or should be supervised by someone who holds the certification. These certificates are accredited and obtained by the City & Guilds and the NPTC or the Scottish Skills Testing Service (Scotland).

A risk assessment must be undertaken to assess who might be harmed and how. Correct selection of pesticide and required method of sheep dipping will enable famers to prevent and control parasites efficiently and to reduce risk of health implications for people involved. A plunge sheep dip is a commonly used method for treating external parasites. This is a permanent structure which involves a trough in the ground, or a mobile steel structure. Mobile sheep dips can also be used. Other methods of applying pesticides include showers and jetters (however, plunge sheep dip compounds are not authorised for use in these facilities). In order to reduce the exposure of contaminants and to protect the environment, the Health and Safety Executive (HSE) suggest that pour on products (to control blowfly and ticks), and injectable treatments for scab control may be used as an alternative to plunge dipping (5). Consultation with veterinary staff is often advised and enables selection of appropriate methods.

The HSE advise the following controls are undertaken to minimise human health and environmental implications:

- Ensuring ventilation, to prevent excessive vapour inhalation,
- Use appropriate equipment such as a metal handed crook and wearing of personal and respiratory protective equipment (RPE and PPE),
- An entry slope, splash boards and screens to reduce splashing from sheep entering the dip bath,
- Ensure that the dip bath has no drains or leaks, and should be inspected prior to use,
- Draining pens should be have an impermeable sloped floor which allows sheep dip compounds to drain into the bath,
- Absorbent material such as sand, earth or sawdust should be used to soak up spillages and placed into a sealed container and labelled for disposal at a licensed waste disposal site,
- Clean water supply for topping up the bath, decontamination and rinsing.

In order to minimise environmental impacts, sheep dips, baths, drain pens and mobile facilities must be located:

- As far away as possible (at least 10m) from a watercourse (6). This includes rivers, streams, ditches, drains, land drains and wetlands.
- At least 50m from any well, spring or borehole (6).
- At least 30m away from any watercourse which drains into a Site of Special Scientific Interest (SSSI). If the farm is located within a SSSI, further advice should be sought from the relevant regulatory body (Natural England, Scottish Natural Heritage, Natural Resources Wales).

Additionally, they should not be situated on or at the top of slopes where there is a risk that spillage might drain directly to a watercourse, or on roads or tracks. Especially in areas of heavy rainfall, where surface run off is often experienced. Pesticides are found more commonly in surface waters due to run off from the direct sources.

**Table 1. Long-term aquatic toxicity data for freshwater organisms exposed to diazinon, relevant to species within the Wyre Catchment.**

| Scientific name | Common name | Taxonomic group | Endpoint | Effect | Test duration | Conc. (µg l-1)1 | Exposure2 | Toxicant analysis3 | Comments |
|---|---|---|---|---|---|---|---|---|---|
| ***Oncorhynchus mykiss*** | Rainbow trout | FIS | NOEC | GRO | 28 days | >200 | f | m | P Unbounded NOEC (highest concentration tested) |
| ***Salmo salar*** | Atlantic salmon | FIS | NOEC | **PHY**<br>**1. Sensitivity of the olfactory system**<br>2. Priming effect of female urine on steroid levels in males<br>3. Effect of female urine on male expressible milt | 4 – 5 hours | **0.1**<br><0.3<br><0.3 | f | m | P;RI 1 |
| ***Anguilla anguilla*** | Eel | FIS | LC50 | - | 96 hours | 80 | ps | pn | P |
| ***Daphnia magna*** | Water Flea | CRU | EC50 | ITX/IMBL | 48 hours | 0.5 | - | - | ***D. magna*** **GM 1.03 µg l-1** |
| ***Acroneuria lycorias*** | Stone fly | INS | NOEC (LC50) | NR MOR | 30 Days | **0.83** (1.25) | NR | NR | P |
| ***Hydropsyche angustipennis*** | Caddisfly | INS | LC50 | MOR | 168hours | 1 | s | m | P |

1The lowest NOECs per group are highlighted in bold font.
2 Exposure: s = static; f = flow-through; sr = static renewal.
3 Toxicant analysis: m = measured; pn = presumably nominal.
ALG = algae; CRU = crustaceans; FIS = fish; INS = insects; ROT = rotifers
DEV = development; GRO = growth; GDVP = general developmental changes; MOR = mortality; PHY = physiology; REPR = reproduction
ELS = early life stage
LOEC = lowest observed effect concentration
NOEC = no observed effect concentration
MATC = maximum allowable toxicant concentration
LC50 = concentration lethal to 50% of the organisms tested
NR = not reported
P = published data
RI = reliability index

# Review of effects of Diazinon on fish species

Moore & Waring, 1996 used electrophysiological recordings to investigate the effects of short-term exposures (30 minutes) to diazinon on Atlantic salmon (*Salmo salar*) olfactory responses to prostaglandin. At a nominal concentration of 1 μg l-1, olfactory responses were significantly reduced compared with controls. The effect was also dose-dependent, with a 10-fold decrease in the sensitivity of olfactory epithelium after exposure to 2 μg l-1 diazinon. The NOEC for this endpoint was 0.1 μg l-1.

The authors then investigated the effects of diazinon on reproductive steroid production and sperm volume following priming with ovulated female salmon urine (Moore & Waring, 1996). After a 120- hour exposure to diazinon, fish were exposed to female salmon urine for a further 3 hours. Fish were then anaesthetised, sperm and blood plasma collected, and an analysis of sperm volume and steroid levels performed. Results from the treated individuals were compared with those of control fish (not exposed to female urine) and primed fish (exposed to female salmon urine). Concentrations as low as 0.3 and 0.8 μg l-1 diazinon caused a significant reduction in plasma 17,20β-dihydroxy-4-pregnen-3-one and gonadotrophin II (GtH-II) levels, respectively, compared with primed controls. Diazinon also affected plasma testosterone and 11-ketotestosterone levels. However, there was no obvious dose response for these endpoints. In addition to the steroid levels, the volume (mg/g body weight) of expressible sperm (milt) was also significantly reduced at the 0.3 μg l-1 level compared with primed fish.

The study demonstrates that the effect of diazinon on olfactory function in fish results in reduced levels of reproductive steroids and volume of sperm. Given the importance of odorants and pheromones in the reproduction of fish, this finding could have serious implications for long-term reproductive success in these fish. This study was well documented and of high quality.

Bisson & Hontela, 2002 investigated the effects of diazinon *in vitro* on the adrenocortical cells of rainbow trout. Cortisol secretion and cell viability were measured in response to diazinon exposure. An EC50 of 233 μM (70.8 mg l-1) and an LC50 of 305 μM (92 mg l-1) diazinon were reported for adrenocorticotropic (ACTH) stimulated cortisol production and cell viability, respectively. The authors suggest the effects on cortisol were probably due to cytotoxicity rather than endocrine effects, as the dose responses for the two endpoints were almost identical.

Macroinvertebrate diazinon tolerance from most tolerant to least tolerant was observed as: flatworms, physid snails, isopods and chironomids (most tolerant); leeches and the amphipod Crangonyx (less tolerant); and the amphipod Hyalella, mayflies, caddisflies and damselflies (sensitive).

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