TRANSFORMATIONS OF DIAZINON, AN ORGANOPHOSPHATE COMPOUND IN THE ENVIRONMENT AND POISONING BY THIS COMPOUND

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Abstract

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Diazinon, an organophosphate insecticide, is a potent blocker of acetyl-cholinesterase (AChE) in the blood and in tissues. Diazinon is commonly used as an insecticide in agriculture, home and gardens. Possible poisoning of the environment by the transfer of the compound to the underground water should be dangerous for water organisms. The rate of diazinon decomposition depends on physical properties of the environment. Especially sensitive to diazinon are insects, aquatic organisms and birds. Mammals are quite resistant to this insecticide. LD_{50} doses differ from 120 ng/insect to 120 mg/kg B.W. for mice. It is a potent blocker of acetyl-cholinesterase (AChE) in blood and tissues. Diazinon is decomposed by oxidation, desulphuration and/or coupling with carbohydrates, amino acids or glutathione to diazoxon or 2-isopropyl-4-methyl-6-hydroxyperimidine (IMHP). From these two compounds diazoxon exhibit higher toxicity than diazinon, while IMHP is non-toxic. In processes of detoxification are engaged cyp and pon-1 proteins. These transformations occur in the microsomal endoplasmic reticulum and are catalyzed by monooxygenases, a group of isoenzymes coded by genes (CYP2C11, CYP3A2, CYP3A4 CYP2D6 in human and CYP2B1/2 in rats). In animal bodies, apart from cytochrome P_{450}, NADPH/cytochrome P_{450} reductase and phosphatidylcholine also take a part in this process. Pon-1 (paraoxonase) activity is crucial for sensitivity or resistance of the organism to organophosphates. Babies and toddlers are particularly sensitive to diazinon. The annual use of diazinon in US is estimated for 30 000 tons. The possible toxicity of this compound to the environment resulted in the decision of EPA to remove diazinon from the use. The phase-out started in 2002 and should be completed to the end of 2004. According to European Community rules the use of diazinon is still possible. It is on the list of active biological substances permitted for use in agriculture in 2004–2010, but not for household use.

Key words: diazinon, toxicity, environmental pollution, decomposition

Introduction

Organophosphate insecticides, such as diazinon, act upon an animal's body by bonding cholinesterase molecules (specifically and non-specifically) both, in blood and tissue. As a result of bonding with the organophosphate compound the acetylcholinesterase (AChE) molecules are competitively blocked, and this process is difficult to reverse. Termination of the activity of AChE takes between a few minutes to over two weeks, depending on the specifics of the organophosphate compound, its dose and the type of tissue. Kamal and Al-Jafari (2000) have studied kinetic parameters of AChE inhibition by diazinon. New method of organophosphate detection was based on their inhibitory effect on AChE using specific biosensors.

Decreasing the activity of AChE causes a termination of hydrolysis of acetylcholine (ACh) molecules to inactive choline and acetic acid in the animal's nerve connections. Diazinon through their phosphate residues, permanently bonds with serine's hydroxyl group, occurring in the catalytic center of AChE. The inactivated esterase, which is formed as a result of this reaction, shows around 10^7 more durability compared with physiological ligands. Each nerve impulse, which reaches synapses with an inactive AChE, causes a synthesis of new aetylocholine molecules. This leads to a permanent blocking of the synapses and the occurrence of disturbances in conduction of nerve impulses. Such mechanism results in the high toxicity of organophosphate compounds, and frequently it is highly increased by impurities in the active product. The presence of these impurities is caused by imperfections of product-making technology, but they can appear also in properly synthesized and purified compounds as a result of extended storage under inappropriate conditions.

Organophosphate insecticides exhibit high affinity to lipids of biological membranes, which facilitates their penetration of insects' cuticulae. This is crucial for the effectiveness of penetration of these compounds into the insects' bodies. Diazinon is a contact insecticide, as its concentration in plants' leaves and stems does not reach sufficient toxicity in insects. The use of this compound to control a wide variety of insects results from its substantially closer relationship with insects' esterases than that of animals.

Diazinon's chemical name is (O,O-diethyl-O-(6-methyl-2-(1-methylethyl)-4-pyrimidinyl) phosphorothioate. The compound's molecular weight equals 304.36. It is a clear liquid (color ranges from amber to brown), well soluble in organic solvents and lipids but only to a small degree in water. Diazinon (Diampilat) is used in products such as Neocidol, Biocide, Spectracide, AG 500, Sarolex, Diazinon and Basudin. It was first registered in the US in 1952. Currently there are over 500 diazinon-containing products on the world market in which it occurs as a single compound or combined with other insecticides such as: lindane, disulfoton and synthetic pesticides known as pyrethroids. They are used for protection of plants, animals and people from insects such as flies, gadflies, fleas, ticks, lice, bird lice, scavenger yellow jackets, itch mites, ants, and many others. Diazinon products are available as dust, powders, granules, seed dressings, wettable powders, emulsificable solutions, oil solutions, and microcapsules. Apart from granules and emulsions in plant production, and powders and aerosols in households, diazinon is used in veterinary products in forms of

aerosols, emulsions, ear tags, and flea collars. The use of diazinon in protecting of plants from pests is very common in Europe, Australia and the United States. During this decade the annual use of this product in the US alone amounted to over 30 000 tones. Diazinon transformations occur in the entire ecosystem: in soil, atmosphere, water environment, plants and animals. European Committee for Environment, Public Health and Food Safety has created major walves in European Parliament debates on the use of possibly hazardous compounds, among others organophosphates. The report was voted 576 votes for, 48 against and 13 abstention for further use of three products from the organophosphate pesticide family: chlorpyriphos, diazinon and malathion in all their uses till 2010 (EPHA Environment Network, 2005).

Soil

Diazinon decomposes slowly in the soil. The pesticide's biological half-life in sterile, neutral soil equals 6–12 weeks, and in soil with bacteria present 2–7 weeks. These differences depend on the type of soil and its moistness. In soils at acidic pH and at high temperatures the pesticide's metabolism is accelerated. Organic substances in the soil may react with the pesticides. Up to 50% of diazinon or its metabolites may be bonded by the soil components, mainly by humin acids, but also by other stable organic and mineral compounds. Increasing quantities of organic substances significantly raise the sorption ratio of the soil. However, the use of surface-active anion substances such as sodium dodecyl sulphate (SDS) decreases the likelihood of sorption of diazinon and its metabolites by the soil. Its principal metabolite is 2-isopropyl-6-methylpyrimidin-4-ol, which has a substantially longer half-life than diazinon itself, and it is also significantly less toxic (US EPA, 1988). In further transformations, the split of the isopropyl and the methyl radicals occurs. Under the influence of soil bacteria the heterocyclic ring breaks down, and the bacteria in its metabolic processes use the carbon atoms. However, only a few percent of diazinon are transformed into CO_2 in the soil. Earthworms exposed to soil with added diazinon have shown symptomatic effects characteristic of AChE inhibition including weight loss, reduced burying ability and curling.

Spraying vegetables with a diazinon-based product it was found that after rain the pesticide's concentration in the surface layer was 2.2 mg/kg of soil. Liquid products are most often used in vegetable production; while in lawn protection, apart from water-based solutions, granulates containing usually 5% of diazinon are also used. The manufacturer's recommended dose oscillates between 0.5 g and 10 g of granules for each 10 m^2 of lawn, depending on the type of insects present in the given area. This form of diazinon is convenient for prophylactic and allows the application of significantly smaller amounts of pesticide. In general, the soil's organic compounds largely bond diazinon, its largest concentration occurs in the topmost layer of soil, and only a slight percentage of diazinon and its metabolites reach the ground waters. The application to the soil of carbon-rich wastes, especially with a high degree of maturity, leads to reduction of pesticide leaching. Another potential fate for pesticide is its volatilisation into atmosphere.

Atmosphere

Diazinon is released into the atmosphere not only during the spraying of plants or using aerosols on animals, but also as a result of evaporation from both, the ground waters and soil. Small amounts of diazinon may also be released by plants, for example during composting (around 0.2%).

In the air the pesticide occurs in the form of vapour with an estimated half-life of 4 hours, as well as in the form of fine powder. In the air it is quickly subjected to transformations caused by UV rays. These reactions are accelerated in conditions of higher temperature and humidity. During the monitoring tests carried out in the US in 1970, diazinon was found in 61% of almost 1000 air samples tested. The average concentration equalled 3 ng/m^3, the highest – 62 ng/m^3. During similar tests carried out in 1999 diazinon concentrations of up to 20 ng/m^3 were found in the air. Diazinon reacts with hydroxyl radicals especially at elevated air temperatures. Such data may provide important information for assessment of the potential of airborne pesticide risks for ecosystem. In the air and rain about 5 to 10-times higher concentrations of pesticides were observed in agricultural than in urban sites. About two decades since DDT was banned in USA in every sample of air from agricultural sites metabolites of this pesticide were still detected.

Water environment

Diazinon is characterised by its high durability in the water environment. In water of neutral pH and of temperature of 20^{o}C the pesticide's half-life equals around 6 months. Altering of pH causes shortening this time so, that at pH 5.0 diazinon's half-life equals 31 days and at pH 9.0 – 136 days. Its faster metabolism in an acidic environment makes diazinon an exception among other organophosphate pesticides.

Diazinon is in the large part bonded in soil, but it can migrate to ground waters, particularly if torrent rain occurs after its application. During a simulation it was found that if heavy rainfall occurs within 2 hours after application of diazinon, the pesticide's concentration in runoff water reaching the concentration around 0.6 mg/dm^3. Following diazinon application at a strawberry farm, 0.15 mg of diazinon was found in 1 dm^3 of rainwater.

Even after 2 months of composting the plants containing around 5 mg/kg of diazinon, it was found that over 1/3 of this pesticide or its metabolites were water-soluble. Due to the likelihood of diazinon contamination, its common use in fighting insects in sheep is a significant threat to the water environment. Simultaneously it was found that a high concentration of zinc sulphate in water decreases the effectiveness of these operations (Higgs et al., 1998). The common use of diazinon caused the fact that in the US its presence has been detected in over 6,000 of 22,000 surface water samples tested, with the highest concentration reaching 33 mg/dm^3. The seasonal variations of diazinon's concentration in surface waters have been observed to have depending on its use. The highest concentration was stated between May and August. In 3,600 water samples taken from deeper layers of soil diazinon had been detected in 74, and the highest concentration was 84 ng/dm^3 (US EPA, 1988). The LD_{50}

(lethal dose for 50% of specimens) value for plankton is quite low, and equals around 0.5 mg/dm^3, which means that this pesticide is highly toxic to water invertebrates. In the cases of diazinon concentration of 27 mg/dm^3 dead cells were found to appear in fish (*Oryzias latipes*) embryos (Hamm et al., 1998). In the natural environment symptoms of toxic effects of pesticides on water invertebrates are quite frequent, as a result of combined simultaneous contamination with numerous compounds (Nohara, Iwakuma, 1996).

Diazinon's substantial durability in the water environment and its high toxicity in fish (cumulation factor of up to 150) are the product's evidently negative attributes. It has been found to be highly toxic to crustaceans, water insects and also toxic to fish (U.S.EPA, 1986). Some species of fish, such as the blue gill sunfish, are particularly sensitive to this pesticide's presence, and their LD_{50} value equals only 70 µg/dm^3. It has been found that even if the pesticide's concentration in water does not exceed 30 µg/dm^3, after 24 hours structural changes begin to appear in the gills, which in turn decreases the gas exchange. Such structural damage of gills exhibited increased thickness of the microridges on gill arch and gill filament, lamellar swelling and reduced microridges. Khalaf-Allah (1999) in experiments performed on *Tilapia nilotica* fish have shown that pesticide water pollution even in low concentrations cause decrease of mean red blood cells and white blood cells counts, haemoglobin, mean cell volume and other haematological parameters. In experiments on European eels it has been discovered that within hours of applying diazinon in concentration of only 42 µg/dm^3 it causes a significant decrease of cholinesterase activity (70–90%) in plasma, brain and eyes (Ceron et al., 1996). In tests performed on young basses it has been found, after a one-day exposure to diazinon in concentration of 90 µg/dm^3, that even such low concentration of the pesticide in the environment causes the drop of cholinesterase activity in the brain (Pan, Dutta, 1998). Much higher concentration of diazinon (2.5 mg/dm^3) killed 1-year old carps after only 4 h, without any influence on brain AChE activity (Dembele et al., 2000). It suggests that brain AChE activity may be a good indicator of chronic pollution but not acute intoxication. This must certainly have a significant impact on lowering the survival capability of those specimens at risk from exposure to the pesticide. In case of 96-hour exposure to diazinon the LD_{50} dose for these fish equals 0.50 mg/dm^3. For rainbow trout the LD_{50} value equals 0.64 mg/dm^3. Lake trout seems to be more resistant to diazinon water contamination (particularly when the water is harder). Diazinon-exposed trouts exhibited decrease in distance swam, speed and turning rate compared to controls. Such behavioural responses provided measures of neurotoxicity and are easy for monitoring. In 2-day tests it has been found that the LD_{50} value for a Japanese predator fish equals 4.4 mg/dm^3, but in the same conditions the LD_{50} value for diazinon's metabolite – diazoxon equalled only 0.22 mg/dm^3. The cumulation factor of diazoxon in the fish's body equalled only 0.5, while the value for diazinon itself reached 50. Diazinon's discharge from these fish's organisms is quite fast, and equals around 0.12, while the same factor for Malathion reaches 0.27. Some fish that live in warmer waters, such as ornamental fish, are much more resistant to a higher concentration of this pesticide, and their LD_{50} dose equals up to 15 mg/dm^3.

Hamm and Hinton (2000) have shown that the exposure of embryos of medaka (*Oryzias latipes*) to diazinon resulted in decreases in hatch success, swim bladder inflation and total

length of larvae. The severity of embryotoxicity was positively correlated with duration of this exposure. Monitoring programs conducted in 1991–1994 in Sacramento-San Joaquin river basin have shown possibility of reduction of invertebrate populations. Diazinon was there used mainly as dormant spray on almonds and other tree crops. Invertebrates (i.e. Daphnia, Ceriodaphnia) are especially sensitive to diazinon and may be subject of acute toxic effect of organophosphates in agriculturally dominated streams and drainage channels as well as in municipal effluents of storm-water discharges. Diazinon 48-h LD_{50} value for Cerodaphnia dubia is only 0.36 μg/dm^3 (Bailey et al., 2001). Fish in these rivers are not at risk from direct effects of diazinon in the water. Ecological damage that may occur is practically limited to Cladocerans.

Plants

As a result of prophylactic use of diazinon in protecting lawns from insects 5–9 mg of this pesticide has been found in 1 kg of grass. Compost produced from this grass contains 0.01 mg of diazinon per 1 kg of grass. Over 30% is transformed into 2-isopropyl form is a much more potent AChE inhibitor than diazinon itself. Another form of oxidation is hydroxylation reactions. Oxidising agents also cause the hydroxylation of the methyl group attached to the heterocyclic ring. As a result of these reactions respective alcohols are synthesised, and as a result of further oxidation – aldehydes and carboxylic acids. Products, which are fragments of the active pesticide's particle, created as a result of these transformations, may be used by plants as material for synthesis of such compounds as amino acids. Parts of a chain together with their active groups may be subject to coupling reactions with simple sugars, oligosugars, amino acids or glutathione. It is also possible for both, the untransformed pesticide and its metabolites to bond with the plant cell walls. The result of these reactions is the so-called pesticide's bonded residue. Other types of reaction occur in the plant surface. In leaves sprayed with a diazinon-containing product it has been found that its concentration exceeds 60 mg/kg. As a result of UV irradiation and heat energy intermolecular rearrangement may occur, which should result in the alkyl residue joining to the phosphorus atom being bonded with an atom of sulphur. The derivatives formed are usually more toxic than diazinon itself. Such transformation also occurs during long-term storage of this pesticide, particularly when stored at higher temperatures.

Insects

Diazinon transformations in insect are usually based on breaking the ester bond (between the oxygen atom and the heterocyclic ring) resulting in 2-isopropyl-6-methylpyrimidin-4-ol formation. Hydroxylation of the isopropyl radical is also possible – as a result of this phosphorothioates are formed. These metabolites differ each from other in terms of toxicity. Whereas the first one is not very toxic to animals, phosphorothioates are a lot more toxic

than the original insecticide. The pesticide's analogues, formed in hydroxylation reactions, easily bond with sugars, glutathione and with glucuronic acid.

One should stress diazinon's closer relationship with the AChE of insects, compared with analogue enzymes of mammals. This fact leads to great differences between the LD_{50} dose of diazinon for animals and insects. For mice the LD_{50} value of this pesticide equals 120 mg/kg B.W. and for houseflies – 120 ng/insect. In order to increase the pesticides' effectiveness, compounds displaying synergetic effect are added to them. Synergetic compounds are not usually pesticides themselves, but they could cause the rise of pesticides' toxicity and often also widen their range of uses. In the case of diazinon the compound used is tridiphane, which –through its emulation with glutathione– terminates the activity of the glutathione S-transferase, which catalyses the reaction of glutathione bonding with diazinon metabolites. By adding tridiphane to diazinon not only a smaller dose of the pesticide may be used, but its range of uses is also expanded. The development of insects' resistance to pesticides is mainly based on acceleration of coupling of these pesticides or their metabolites with glutathione and sugars. On the other hand, the synergistic' activity is characterised by little selectivity in terms of animal enzymes, which causes the slowing down of transformations not only of the pesticide itself but also of other reactions catalysed by the same enzymes. The 2,4-dinitro-1-chlorobenzene has similar properties to tridiphane.

Changes of the enzymes activity taking part in diazinon transformations are caused not only by the increase of the enzyme: substrate relationship but also by changes in the enzyme synthesis caused by genetic factors. As it has been found in blowfly an ectoparasite of Australian sheep, that changing one amino acid in the enzyme coded in the ROP-1 gene causes a change of the enzyme's qualities from esterase to a hydroxylase specific for diazinon. A similar mechanism was found in the housefly, in which substitution of glycine-137 for asparagine in protein chain changes the function of this enzyme from esterase to a hydrolase (Claudianos et al., 1999). The LcαE7 gene, as well as MdαE7 gene, which cause the insects' increased resistance to pesticides are responsible for these changes. It seems that this resistance is limited to only one pesticide, which means that probably one gene is responsible for its occurrence. The Australian sheep blowfly, resistant to diazinon, but not malathion, display a low activity of enzymes involved in methyl butyrate hydrolysis ("ali-esterase"), high activity of organophosphate hydrolysis and low activity of malathion carboxyl esterase, compared to the control group of insects. Insect's resistance to Malathion have on the other hand been found to have a high activity of this last enzyme (Campbell et al., 1997). It is possible for insects to develop a genetic mechanism for organophosphate pesticide resistance based on the intensification of the synthesis of selected cytochrome P450 isoenzymes, responsible for the biotransformation of these pesticides. It has been found that higher resistance to organophosphate pesticides depends on the activity of the monooxygenase system, the synthesis of which is coded in the CYP6A2 (Bride et al., 1997) and/or CYP6A1 genes (Sabourault et al., 2001). An increased activity of the cytochrome P_{450} isoenzymes, which raises the insects' tolerance to diazinon, may be caused by prior use of phenobarbitone, which increases P_{450} cytochrome synthesis. It was shown that 10 years of using organophosphate insecticides in ear tags caused not only increased resistance to

used insecticides but also increased activity of AChE observed in field population of horn fly. Also comparing the effectiveness of diazinon and new formulation of deltamethrin used in dogs (in impregnated collars) to control fleas it was noticed, that new formulation was much more effective (Franc, Cadiergues, 1998).

Aquatic life is very sensitive for organophosphate insecticides. These chemicals are frequently detected in nearby surface waters and in rivers and lakes. The concentration of these compounds in water strongly depends on a number of parameters such as the amount of used compounds, soil structure, soil sorption/desorption processes (Cooke et al., 2004), migration of the insecticides to the surface water, intensity of rains and compound decomposition in the environment. All these factors may lead to occasional, seasonal substantial increase of organophosphates over EPA allowed concentrations (Phillips, Bode, 2004). Conners and Black (2004) tested the toxicity of copper and some of fertilisers, herbicides and insecticide routinely used in care and maintenance of lawns. The most toxic pollutant for Utterbackia imbecilis was copper (LD_{50} – 37.4 μ/dm^3) while the Mussel glochidia were less sensitive for diazinon as compare to all other chemicals examined. This compound exhibits moderately strong soil adsorption (K (d) 12 – 35) and low sorption reversibility (<15%) (Cooke et al., 2004).

The concentration of diazinon, in environmental matrices, among others, depends on its decomposition. In aqueous phase degradation of diazinon is a fast process. The mechanism of its degradation is probably based on hydroxyl radical attack, resulting in the substitution of sulphur by oxygen, S bond cleavage of the pyrimidine ester bond and oxidation of the isopropyl group resulting in the formation 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP) (Kouloumbos et al., 2003), which is less toxic than diazinon.

Diazinon transformations in animals, similarly as in plants, are mainly based on enzymatic processes of oxidation following coupling of the derivatives formed with amino acids, glutathione or carbohydrates. Diazinon should be metabolised in two major pathways: to diazoxon through CYP metabolism and to IMHP. Diazoxon may be detoxified to IMHP by further cyp, as well as pon-1 metabolism (Poet et al., 2003). These transformations occur in the microsomal endoplasmatic reticulum and are catalysed by monooxydases. These enzymes display low specificity regarding the substrate and react with many xenobiotics. This is made possible by the fact that cytochrome P450 isoenzymes are very numerous, and each one shows a similar relationship with the substrate. In experiments carried out on hepatocytes of rats it was found that these isoenzymes are coded by the genes CYP2B1/2 (rat) (Fabrizi et al., 1999), CYP2C11, CYP3A2, CYP3A4, CYP2D6 (human) (Tang et al., 2001) and the synthesis of the enzymatic protein is increased by such stimulators as phenobarbitone and dexametasone (Abdelsalam, 1999). In the animals' bodies, apart from cytochrome P_{450}, NADPH/cytochrome P_{450} reductase, phosphatidylcholine also take part. The O-dealkylation reaction, which is based on breaking the bond between the phosphor and oxygen atoms, is also important. This is an ester type of bond, and as such may be broken by esterases in the hydrolysis reactions. Apart from esterases, other enzymes, such as monooxygenases and glutathione S-alkyltransferase, may also catalyze these types of reactions. Since this pesticide's metabolism is quite complicated, the transformations of

many other xenobiotics may influence the effectiveness of diazinon biotransformation in animals. Administering organochloride compounds (i.e. aldrine) to the animals stimulates the activity of liver esterases, while DDT increases the activity of the monooxygenase system. The effects of pesticides or their metabolites are based on inhibition of liver monooxygenases activities. A good example is the inhibitory effect of sulphur, which is released from diazinon as a result of the desulphuration reaction, on the activity of the cytochrome P_{450} (hydrodisulphide of the cytochrome P_{450} is formed in this reaction). A result of cyp- and pon-1 mediated metabolism is activation of diazinon to diazoxon and detoxification to IMHP. Pon-1 (Paraoxonase) is an A-esterase and the activity of this enzyme differs among animal species. Birds exhibit very low activity of pon-1, while rabbits very high. The highest activity was found in liver and plasma. This enzyme is crucial for sensitivity or resistance of the animal to diazinon. Animal with low activity are more sensitive to applied insecticide. Catalytic efficiencies of pon-1 to hydrolyse oxon substrates predict the degree of in vivo protection against applied of sulphur organophosphates, such as diazinon (Costa et al., 2003). In reactions are engaged intestinal and hepatic microsomes. Numerous microvilli designed to maximize surface area of the intestine increase absorption of the gut content, including organophosphorus insecticides. In intestinal enterocytes diazinon is actively metabolized by both cyp- and pon-1 enzymes prior to entering the liver via blood circulation system. The activity of these enzymes in enterocytes, as well as in the liver, should be responsible for metabolism of diazinon, toxic xenobiotic compound, and finally for its concentration in the organism resulting in the inhibition of AChE in synapses. The basic reactions of diazinon transformations in the bodies of mammals, similarly as in plants, are based on the oxidizing desulphuration and hydroxylation reactions. After the O-dealkylation reaction analogues are formed, which constitute the main forms of diazinon found in the urine of dogs (Agency Toxic Subst. Dis. Reg., 1996): 2-isopropyl-6-methylpyrimidin-4-yl, 2-(2'-hydroxyisopropyl)-6-methylpyrimidin-4-yl and 2-(1'-hydroxyisopropyl)-6-methylpyrimidin-4-yl. In the case of diazinon, the hydroxyl derivatives may however be significantly more toxic to mammals than the original pesticide. This occurs in cases of some hydroxyl analogues. It has been found that for mice the LD_{50} dose of O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate equals 120 mg/kg B.W., while this dose for diazinon itself equals 300–850 mg/kg B.W. However in most cases the pesticides' hydroxyl derivatives are much less toxic than the pesticides themselves, as those analogues are more easily coupled with carbohydrates. As a result of these reactions the appropriate α-O-glycosides are formed. Coupling of diazinon metabolites with glutathione is in birds and mammals one of the basic reactions of detoxification of this pesticide. Enzymes from the glutathione S-transferase group exhibit a high activity in the microsomal system of the liver. As a result of these reactions appropriate conjugates of glutathione with diazinon metabolites are formed.

Diazinon's toxicity in birds

Compared with mammals, birds show a much higher sensitivity to diazinon's toxicity. This is why in 1998 the US Environment Protection Agency has issued restrictions regarding

the use of diazinon in open spaces. The restrictions apply to golf courses and turf-growing farms. Diazinon caused accidental poisonings of 23 different species of birds, and in cases of Atlantic geese and Canadian geese contributed to a decrease of population. It has been accepted that for birds the LD_{50} dose varies between 2 and 40 mg/kg B.W. Oral LD_{50} dose for a Mallard duck equals 3.5 mg/kg and for a quail – around 10 mg/kg B.W. Such poisonings also took place on farmlands. A mass poisoning of pigeons took place at a strawberry farm in British Columbia after torrid rain. The pesticide's concentration in the stomachs of the dead birds was performed a substantial part of the pesticide had been absorbed. The activity of cholinesterase in the brains of the dead birds equaled 2.25, while in healthy birds 7–10 mol/min/g, respectively. Tests carried out on ducks showed that administering various organophosphate products, including diazinon, in toxic doses, lowers the activity of cholinesterase in the brain by 70–80% in the period of 24 hours after application (Environmental Health Data Search)**.**

Diazinon's toxicity in mammals

Diazinon's biological half-life in mammals equals about 12 hours, and after 2 weeks only traces of this pesticide may be found in the bodies (Environmental Health Data Search). As experiments carried out on rats and rabbits demonstrated, diazinon has a low to moderate toxicity in animals. In oral application the LD_{50} for rats varied between 66 and 635 in females and 96–967 mg/kg B.W. in males. After an oral application of diazinon (80 mg/kg B.W.) it has been found that it is quite quickly discharged from the rats' organisms, and after 2.9 hours its concentration in the body fell by 50%. In plasma almost 90% of the pesticide administered occurs in the form bonded with proteins. After an intravenous application of 5–10 mg of diazinon per kg B.W. its elevated concentration has been found in the rats' kidneys, livers and brains (Extoxnet).

The relations between the dose of diazinon and its effect on neuromuscular transmission were studied on mouse model by de Blaquiere et al. (2000). They have shown that repeated doses of diazinon produce long-term electrophysiological changes of neurotransmission in the mouse. Low doses of this pesticide had no effect on AChE activity in the brain and soleus. The oral application of diazinon to rats resulted in changes of cholesterol metabolism (Ibrahim, El-Gamal, 2003). The effect was dose-dependent and may be summarised as causing the increase of LDL and triacylglyceroles (TAG) and significant decrease of HDL and phospholipids (PL), which may suggest, that diazinon may interfere with lipid metabolism in mammals.

Tests carried out on rabbits using diazinon in the form of an aerosol showed a decrease of activity of fast response receptors in the lungs, which may have an inhibitory effect on the lungs' immune system when rabbits were given 1:10 diluted Diazinon PLUS. It has been found that diazinon is much less toxic in the cases of skin contact. In rabbits the LD_{50} dose of diazinon applied directly to skin equals 3.6 g/kg B.W. Direct application of diazinon to the skin of cattle causes that its traces appear in the milk 1 day after administration. Diazinon had been entirely discharged from a cow's organism within 2 weeks. It has also been found that cats show a much higher susceptibility to diazinon poisonings than dogs.

Chronic poisoning by this product occurs if its intake exceeds 10 mg/kg B.W./day in pigs and 1000 mg/kg B.W./day in rats. In sheep an oral application of diazinon in doses of 50 mg/kg B.W. for 21 days did not cause any significant clinical symptoms or pathological changes aside from decreasing the cholinesterase activity (Greenman et al., 1997; al-Qarawi et al., 1999). It was found that after extended use of feed with an addition of diazinon the cholinesterase activity in the erythrocytes, plasma and brain tissue decreased. On the other hand, Hatjian et al. (2000) reported that erythrocytes AChE and plasma or serum acetylcholinesterase activities were almost unchanged after exposition of the sheep to dip containing diazinon. After in vitro experiments carried out on cell cultures of intestinal epithelium of rats and humans it was found that diazinon concentration of up to 15 mg/dm^3 of medium increases the proliferation of these cells. Qiao et al. (2001) have shown in vitro, that diazinon inhibited DNA synthesis preferentially in C6 gliotypic cells. It means, that this pesticide has immediate, direct effect on neural cell replication.

Based on research carried out on rodents it seems that diazinon is not carcinogenic. In rats fed with fodder containing an addition of 45 mg/kg B.W./day of diazinon for 2 years no increase in susceptibility to cancer has been detected.

Results of tests concerning diazinon's teratogenic effects are not univocal. Injections of diazinon into hens' eggs caused deformations to the skeleton and the spinal cord of the embryo, while in quail it only resulted in disturbances of skeletal development. Low daily doses of 0.125–0.25 mg/kg B.W. did not affect the embryo development of hamsters and rabbits. High doses such as 1–10 mg/kg B.W./day halted the growth of embryos of both pigs and dogs (Iverson et al., 1975).

Similarly as with other mammals, diazinon's toxicity in humans is also low or moderate, depending on the character of the product used. The transformation of diazinon to diazoxon in human liver was described. The K(m) value of diazoxon formation varied markedly among subjects. Putative high-affinity component correlated with S-mephenytion 4-hydroxylase activity suggesting the involvement of cyp2c19. From different human P_{450} enzymes cyp2c19 activated diazinon at the fastest rate followed by cyp3a4 and cyp1a2 (Kappers et al., 2001). In tests carried out on humans it has been found that diazinon is rapidly discharged from the system with urine and faeces and after 12 days only its traces remain in the organism (Lewis et al., 2001). The product is easily absorbed through the skin. Consumption or inhalation of diazinon cause the appearance of following symptoms: headaches, discomfort, speech disorders, feeling of weight on the chest, breathing difficulties, excessive salivation, serous secretion from the nasal fossa, constricted pupils and lack of reaction to light, seeing in red and blue, weakness, stomach cramps, nausea, vomiting, diarrhoea and muscular trembling. In cases of more sever poisoning coma and damage of liver and kidneys occurred. The lethal dose of diazinon for humans was found to be 360 mg/kg B.W. As a result of chronic poisoning by this organophosphate in addition to the above-mentioned symptoms dysfunction of the frontal brain lobe has been found.

Babies and toddlers are particularly sensitive to diazinon poisonings (al-Qarawi et al., 1999). In one case of inappropriate use (against the manufacturer's recommendations) of diazinon solution in the entire house (even some furniture had been sprayed) a 3-month old

baby's urine had been found to contain a high quantity of diazinon metabolites, comparable with that which was characteristic at farmers repeatedly using this product. In adults the concentration of these metabolites was undetectable. Wilson et al. (2001) found in air, food beverages and soil samples obtained in child day care centers low levels of pollutants (below the levels considered to be of concern as possible health hazards). They noticed that potential exposures through dietary ingestion were greater than those through inhalation. It is likely that diazinon could largely enter into the baby's system by contact. Simulation results indicate that children's handling of the food could contribute from 20 to 80% of total dietary intake of pesticides. Other possible way to intake diazinon is the contact of the food with household surfaces sprayed with insecticides. The amount of transferred toxin depends on the kind of surface, time of contact of the food with the contaminated area and on the force of the contact. In extreme conditions (60 min at force of 1500 g) up to 83% of the toxic insecticide should be transferred to the food. Mean transfer efficiency was estimated for 15–70%. Additionally, babies inhale more air, with respect to their metabolic mass, compared with adults, which is particularly important in using the aerosol forms of this product. Doctors have found excessive muscle tension in the baby's legs; this symptom disappeared 6 weeks after leaving the house in which the excessive concentration of diazinon was present. The increased sensitivity to poisoning by pesticide is common not only in children, but has been found also in young animals (U.S.EPA, 1988). Other experiments have demonstrated that even few months after using of diazinon in households it is still possible to detect its metabolites in the residents' urine (Wagner and Orwick, 1994). Using very sensitive method of pesticide estimation – isotope dilution HPLC/MS Baker et al. (2000) detected diazinon metabolites in 57% of urine samples from patients without documented pesticide exposure. The potential development and physical adverse effects of chronic exposure have increasing concern. Based on experiments conducted on animals it seems that diazinon should not be carcinogenic to humans. However in tests on human lymphocytes it has been found that a 0.5 mg/dm^3 concentration of diazinon causes changes in chromosomes. (Wu et al., 1996). Despite diazinon's merits such as the relatively low dose used to fight parasites, lower durability and the low potential for cumulating in the bodies of mammals and all its insecticides benefits we cannot separate it from its influence on ecosystem. Overall research shows common use of diazinon and that its influence is globally widespread to all environmental media. In general, this organophosphate is degraded fairly rapidly in natural settings, however some products are at least as toxic as the parent compound. Biological fate of this pesticide is complex, mediated mainly by diverse metabolic mechanism. Because this pesticide is so popular its use leads to increasing of insects' resistance and to keep the effect on the same level we have to use higher doses (so called pesticide treadmill). Its highly lethal effect on aqueous invertebrates and birds need explanation. There are also suggestions that unregulated setting inside the buildings and in the close neighbourhood may create substantial risk for humans – especially for children. First restrictions of diazinon use in golf courses and sod farms are important but there still exist widespread avian exposures from orchards and lawns. It is critical to use it with great care to minimize its negative impact to ecological systems.

These data should be the reason of EPA decisions concerning the limitation of the indoor use of diazinon following phase-out of lawn and gardens use started from 2001 (EPA, 2000a) in USA. For indoor use registration was cancelled on March 2001 and sales will stop on December 2002. For lawn and gardens use the dates are August 2003 for distribution and the phase out of the product was completed in 2004. The program was introduced because of food risks (acute – 63% and chronic – 22%) in 1–6 years old children; water risk (acute and chronic intoxication for water drinkers; occupational risks during mixing and application activities; ecological risks – high toxicity of diazinon to birds, fish and water invertebrates, honey bees and other beneficial insects and wild life (EPA, 2000b). The studies of Carlton et al. (2004) indicated, that still in June 2004 106 stores selling pesticides contained chlorpyrifos (4% of stores) (rejection date Dec. 2001) and diazinon (40% of stores) (rejection date Dec. 2002). The EPA's phase-out looks to not completely effective in the case of diazinon. Similar action as in USA. in Europe is still discussed by the EU Committee for Environment, Public Health and Food Safety.

Conclusions

1. Diazinon, an organophosphate insecticide is a strong environment pollutant.
2. It is toxic not only for insects but also is dangerous for water organisms and mammals.
3. Its stability makes, that its toxic action should be prolonged for a long period of time.
4. Ban of marketing insecticides containing diazinon in USA is showing that it will be necessary to take the same action in countries being EU members.

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