Chapter 9

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Veterinary Medicine and Biomarkers in the Assessment of the Environmental Pollution with Hazardous Waste

1. Introduction

One of the most serious threats to the natural environment is hazardous waste deposited in various forms in the ground. About 340 of such waste disposal sites were created in Poland in the previous century, in which over 10,000 tons of pesticides (among others) were deposited. The aim of those disposal sites, also referred to as pesticide tombs, was to solve the problem of hazardous substance neutralization. However, the disposal sites were, in most cases, established carelessly. Therefore, their exact number and the types of substances inside them is not known. An even more serious problem is securing the discussed disposal sites against release of toxic chemicals to the environment. It has been confirmed that the components can leak into the natural environment and pose a risk of polluting ecosystems located in the direct vicinity of damaged deposits (Amador 1992, Burton *et al.* 2001).

Numerous studies have shown that organisms sensitive to foreign substances (biomarkers) provide the most accurate reflection of environmental pollution with pesticides (Pawert *et al.* 1998, Szarek *et al.* 2000). This thesis is clearly confirmed by the research conducted in the Iława Lakeland, where a floristic and phytosociological assessment of an area with an existing and polluting pesticide tomb and of the natural environment following land reclamation was carried out.

2. Historical outline

Pesticides (from Latin *pestis* – epidemic, plague, *caedo* – I kill) are poisons used to eliminate harmful or undesirable organisms in agrocenoses and during the storage of farm crops. They have also been applied for non-agricultural destruction of harmful organisms, as agents used for disinfection of facilities, wood preservation and pest control in residential premises. The main criterion for pesticide classification is their intended use.

From the historical perspective, it can be observed that the oldest group of plant protection agents are insecticides. It is well-known that the Sumerians repelled insects by rubbing the skin with a preparation containing sulphur as early as in 2500 BC. The „Ebers Papyrus", dated to about 1550 BC and considered to be the oldest medical document in ancient Egypt, describes about 800 recipes used for insect control purposes. In 1200 BC, the Chinese used lime and plant origin insecticides to protect seeds against pests and in about 200 BC also used arsenic products. Modern times, with the development of natural sciences and technology, brought about a more rational approach to plant protection products. Initially, preparations of plant origin were the main agents used to fight harmful insects. These were most frequently preparations obtained from tobacco leaves, pyrethrum flowers (*Chrysanthemum cinerariaefolium*), as well as roots of plants belonging to the *Lonchocarpus* or *Derris* genus. A real breakthrough in pest control occurred in 1939 when Paul Müller discovered the insecticide properties of dichlorodiphenyltrichloroethane (DDT). It was a „miraculous powder", which saved many people during World War II and in the post-war period by eliminating insects carrying malaria and typhus dangerous for people. In the 1950s, it was applied on a mass scale in the belief that this product did not demonstrate any harmful properties for humans, birds or mammals. A few dozen years of DDT application were required to prove that it is very persistent in the environment and highly hazardous. It causes cancer in humans and animals and has a negative effect on reproduction.

Norway and Sweden were the first countries to prohibit DDT application (in 1970) and the USA banned it in 1972. In Poland, DDT was withdrawn from use in 1976. However, the substance was applied in the Soviet Union until 1983. This led to using 70,000 tons of this toxic substance in Uzbekistan, which brought about high pollution with DDT residues in poultry, eggs, powdered milk, soil and water (Gonzales 1999). In 2001, the Stockholm convention was ratified, signed by 98 states committing not to use, or to significantly reduce, the application of non-degradable pesticides. For many years, a negative attitude towards chemical plant protection, symbolized by DDT, has strengthened in social awareness.

3. Pesticide tombs – intended use and structure

Pesticides, which were withdrawn from trade and agriculture for ecological and toxicological reasons, became hazardous waste. The past century left tons of accumulated pesticide waste which, when inappropriately stored, can leak uncontrollably into the natural environment and endanger all forms of life.

Construction of pesticide tombs, in which a significant share of plant protection chemicals were deposited, started in the 1970s. Pesticide tombs most frequently were 3-4 m deep wells, build of concrete rings 1-3 m diameter, or brick constructions, which after filling with pesticides, were filled in with about a 0.5 m thick layer of soil. According to the estimates of the Ministry of Environmental Protection, the total weight of pesticide waste in Poland may be up to 60,000 tons. About 340 pesticide tombs contain 10,000 tons of substances, of which DDT makes up a significant part (Amador 1992, Zaleska, Hupka 1999). Due to the high randomness in the choice of the method and place of pesticide disposal, the precise amount and composition of accumulated plant protection agents could not be determined.

An attempt to make an inventory of pesticide tombs was undertaken in 1993 by the Plant Protection Institute in Poznań, together with provincial inspectors of the Inspectorate of Environmental Protection and the Inspectorate of Plant Health. While designing pesticide tombs in the past, their long term exploitation was not taken into consideration. The dumping sites were located quite at random, without taking into account hydro-geological conditions, characteristics of the surrounding area or environmental conditions. Such an approach resulted in storing pesticides on geological formations of high permeability and sometimes even on water-bearing layers. The inspection carried out by SANEPID, the public sanitary inspection authority, found that 1/3 of the examined pesticide tombs did not satisfy location conditions. However, this does not define the extent of the problem, since it is expected that a significant number of pesticide tombs have not yet been found. The examined pesticide tombs are often located less than 300 m from water intakes, water bodies and farming areas. At least 75 documented pesticide tombs neighbour a river or a lake, while 100 are situated near drinking water intakes and 140 are located near human settlements. It has emerged that pesticide tombs situated in the areas of former state-owned farms are in the worst condition. In most cases, they are simply pits in the ground. The technical condition of those disposal sites is unacceptable and the pesticides leak into soil, water and air over large areas, polluting the ground waters, surface waters and subsoil water bodies in the vicinity of pesticide tombs (Ignatowicz 2007, 2008).

By the 1970s, the golden age of the production of plant protection chemicals was over. The ban on the use of non-degradable pesticides created a problem with what to do with dozens of tons of toxic compounds. Pesticides past their expiration date were usually deposited in concrete underground tanks of various sizes and locations. The majority of them were not suitable for storing hazardous waste. Most often pesticide tombs were insulated with pitch or adhesive, which was not enough to secure the sites against pesticide leaks to the environment (Ignatowicz 2007). In terms of technical structure, pesticide tombs can be divided into three types:

1. ground pits – pose the greatest threat due to the lack of any insulation between deposited pesticides and ground; most ground pits are located in the south of Poland,
2. well rings – those pesticide tombs are 3-5 m deep and 1-5 m in diameter; this type also include concrete tanks,

3. military bunkers – former military facilities or fodder silos used as pesticide tombs.

Examination of the technical conditions of those disposal sites proved that they were unsatisfactory in the great majority of cases. In view of that, pesticide tombs are not safe dumping sites but „pesticide bombs". They have earned such a name because of errors in construction and the application of inappropriate materials for their construction which were prone to corrosion. Additionally, the areas where waste dumps are located are not properly fenced or marked. Fencing constructed at the same time as pesticide tombs was most frequently made of impermanent materials and did not stand the test of time. Warning tables suffered a similar fate. Pesticide tombs can be also classified in terms of their construction and location:

- I hazard category – this category includes pesticide tombs posing the highest threat to the environment – ground pits,
- II hazard category – this group includes pesticide tombs, the location of which is dangerous for water intakes or surface waters, as well as dumping sites located in wet areas or at risk of flood,
- III hazard category – this group includes pesticide tombs releasing pesticides into ground and underground waters, which have not been included in category I and II,
- IV hazard category – pesticide tombs in the form of military bunkers,
- V hazard category – other tombs.

The composition of hazardous waste on the basis of 96 pesticide tombs according to is usually as follows: phthalimides 1%, fourth grade amines 2%, tars 3%, carbemates 4%, nitro derivatives 8%, phosphoorganic 10%, phenoxy acids 12%, ditiocarbaminians 14%, inorganic 17%, chloroorganic 29%. On the other hand, the percentage share of DDT in the entire volume of chloroorganic waste is as much as 42%.

The interest in pesticide tombs as objects posing an unquestionable risk to the natural environment started in the 1990s. It quickly emerged that some of these sites were not recorded in any documents, as administrative changes in the 1970s caused dispersion of both technical documentation and documentation concerning plant protection agents past their expiry date. This is why the location of many pesticide tombs was forgotten.

Consequently, it became necessary to make an inventory for pesticide tombs and to perform an impact assessment of pesticides stored in such dumps on the ground and water environment. In 1998-1999, inspection authorities carried out 108 examinations of pesticide tombs throughout the Poland. These analyses resulted in post-inspection recommendations concerning arrangement of the area of pesticide tombs (mowing the grass, completing fencing and marking) preparing expert opinions concerning the tightness of disposal sites and carrying out soil research. The largest pesticide tombs are located in regions where state-owned farms prevailed. It is estimated that the total amount of plant protection chemicals deposited in such pesticide tombs is about 10,000 tons (Ignatowicz 2007, 2008). No reliable documentation concerning pesticide tombs has been preserved;

therefore, the given value is only approximate. It is estimated that almost 800 informal ground pits still contain up to 12,000 tons of pesticides. It has been proven that toxic compounds in many cases were released from inappropriately secured pesticide tombs to the natural environment and that trace amounts of DDT in water have become multiplied by 10x in plankton, 500x in gammarus, 2,500x in the bodies of small fish and 5,000x in the bodies of predatory fish (Burton *et al.* 2001, Depledge, Fossi 1994, Geyer *et al.* 1997, Graumann, Drukker 1991, Pawert *et al.* 1998, Sawicka-Kapusta *et al.* 1990, Sawicka-Kapusta, Zakrzewska 1998, Schramm *et al.* 1998, Schweiger *et al.* 1997, Szarek *et al.* 1997, 2000, Triebskorn *et al.* 1997).

4. The role of a veterinary doctor in selecting an appropriate biomarker

One of the characteristic features of DDT is its ability to bioaccumulate in subsequent links of the food chain. This is illustrated by the effects of its application near Lake Michigan in the form of the following volumes of residues in the environment: water from the lake – 0.000003 mg/kg, small crustaceans – 0.04 mg/kg, small fish – 0.5 mg/kg, predatory fish – 2 mg/kg, and predatory birds – 25 mg/kg. Such environmental pollution was reflected in a decrease in the predatory bird population. The knowledge of those facts indicates the need to carry out research concerning the impact of pesticides on animals living in such environments. A complex study carried out in the Iława Lakeland illustrates the effect of environmental pollution with pesticides on the surrounding ecosystem. This particular site was thoroughly chosen for the study because of an insufficiently secured xenobiotic dumping site existing there and the proximity of a carp fishpond and a field and forest. Many detailed analyses have been carried out, which explicitly reject environmental quality and quantity studies as a reliable reflection of the impact of environmental pollution on animals. Pesticide concentration in the environment is usually low and variable depending on the climate, type of soil, composition of fauna and flora.

The research carried out in the Iława Lakeland demonstrated that organisms sensitive to foreign substances – biomarkers – are much more useful in this respect (Fabczak *et al.* 2001, Szarek *et al.* 1995). A pesticide tomb located in the research area was situated on a sandy hill at the edge of the forest. It was located about 500 m away from a nearby village, 100 m from carp fishponds, 600 m from a drinking water intake and 750 m from a lake. While liquidating pesticide tombs, it was established that the area featured 32 concrete silos and 2 ground pits not secured in any way, containing dozens of tons of plant protection chemicals (mainly DDT) past their expiry date. Research rodents*:* the yellow-necked mouse (*Apodemus flavicollis*), the field mouse (*Apodemus agrarius*) and the bank vole (*Clethrionomys glareolus)* were obtained from the nearby forest and meadow. Carp fry was caught in the fishpond located 100 m away from the pesticide tomb, while a control sample was collected from a pond located 2 km away from the pesticide dump (Szarek *et al.* 2007).

5. DDT and its derivatives in soil, water and sediments

High levels of DDT and its metabolites were found in soil samples collected from the area remaining after the removed object and the obtained data decreased by 1/5 in subsequent years of research. Four years after removing pesticide tombs, they were recorded as: DDT – 1,894.956 ppm, ∑DDT – 3,099.174 ppm, DDD – 1,064.239 ppm, DDE – 139, 979 ppm. In the control soil, these values were around a few orders of magnitude lower.

Bottom deposits in the examined lake contained mainly DDE and the values in this case reached about 1 ppm, while only slightly lower values were recorded in sediments of the control lake. No pesticides were found in surface waters of the examined lake, but they were found at a depth of 8 m, where they occurred in trace quantities (Szarek *et al.* 2007, Skibniewska *et al.* 2004).

6. DDT and its derivatives in fish

The content of DDT and its metabolites was determined in carp (*Cyprinus carpio*) fillets. The following results were obtained: mean content of ∑DDT in carp fillets from the examined pond amounted to 0.1127 ppm, DDT 0.0569 ppm, DDE 0.0267 ppm, DDD 0.0291 ppm. On the other hand, those values in the case of control carps were as follows: mean content ∑DDT – 0.103 ppm, DDT 0.0493 ppm, DDE 0.0291 ppm and DDD 0.0166 ppm.

While interpreting the obtained data, it was noted that high residues were determined for fish samples which were used for control purposes. The control ponds were surrounded by forests and farming fields, where according to their owners, no crop protection chemicals had been used. However, as it is informally known, DDT was used in 1980s to protect Polish forests attacked by the gypsy moth (*Lymantria dispar*) and could have been the source of pollution over the areas of the examined fish farm. According to various sources, the carrying off range of crop protection agents while applying agricultural aviation treatments can be even 50% of the applied dose (Hayo 1996). It is a well-known fact that environmental pollution by pesticides is an inevitable phenomenon also beyond the area of its application (Pimentel 1995).

It should be mentioned that residues of DDT and its derivatives determined in fillets of the examined carps after removal of the pesticide tomb proved significantly higher than the values found in previous years, which proves the secondary pollution of the area around the pesticide tomb during its liquidation (Szarek *et al.* 2007, Skibniewska *et al.* 2004).

7. DDT and its derivatives in field mice and yellow-necked mice

The data obtained on the content of DDT and its derivatives in field and yellow-necked mice ranged between 0.102 ppm and 0.192 ppm.

It should be emphasized that the first examination after removal of the pesticide tomb revealed a level of DDT and its derivatives in the examined mice that was over twice as high as before liquidation of the pesticide tomb (Szarek *et al.* 2007, Skibniewska *et al.* 2004).

8. Morphological examination of mice

Morphological examinations were carried out on black striped-field mouse (*Apodemus agrarius*, Pallas 1771) and yellow-necked mice (*Apodemus flavicollis*, Melchior 1834) caught in the area of the Iława Lakeland near the pesticide tomb. The research was of a comparative type, it was carried out on mice caught before removal of the pesticide tomb and two years after its liquidation.

Morphological lesions observed in the liver in the presented mice living near the pesticide tomb were characteristic for environmental polluted ecosystem (Fig. 1-4, 7-12). Lesions typically concerned small parenchymal areas of hepatic cells and were located near blood vessels. Parenchymal degeneration, steatosis simplex and degenerative of the liver were sporadically recorded. Circulation disorders, inflammatory lesions and progressive lesions (include binucleate hepatocytes) were relatively infrequent and they occurred most often in animals feeding near the pesticide tomb.

While analysing the degree of intensity of morphological lesion in time perspective, it can be observed that they remained on a similar level in a last period of the pesticide tomb's existence. However, two years after its liquidation, the discussed deviations clearly intensified. This proves that there was secondary environmental pollution during liquidation of the pesticide dump (Szarek *et al.* 2007, Skibniewska *et al.* 2004).

9. Morphological examination of fish

Macroscopic assessment of carps from the pond near the pesticide tomb revealed only hyperaemia in gills and in the hepatopancreas. On the other hand, such lesions were sporadically recorded in fish caught in the control pond. Most morphological lesions concerning the hepatopancreas were observed in fish living in the pond near the pesticide tomb as compared to control carps (Fig. 5, 6). This relationship also concerned parenchymal degeneration. Usually parenchymal degeneration occurred in small areas of parenchymal hepatic cells and was frequently located near blood vessels. The lesions were of limited nature, and parenchymal degeneration often covered only a few hepatocytes. Lesions in mitochondria and rough endoplasmatic reticulum ultrastructurally were quite often observed, as it was place in mice.

In carp kidneys, irrespective of fish origin, parenchymal degeneration of ductular epithelial cells was often observed and hyaline droplet degeneration was sporadically recorded. Both the occurrence of morphological lesions in kidneys and the degree of their intensity were similar in fish bred in the pond near the pesticide tomb and those in the control pond.

The research proved that the most significant differences in morphological lesions concerned the hepatopancreas of carps living near the pesticide tomb in comparison to fish originating from the control pond. This observation concerned both the quantity of lesions and the degree of their intensity (Szarek *et al.* 2007, Skibniewska *et al.* 2004).

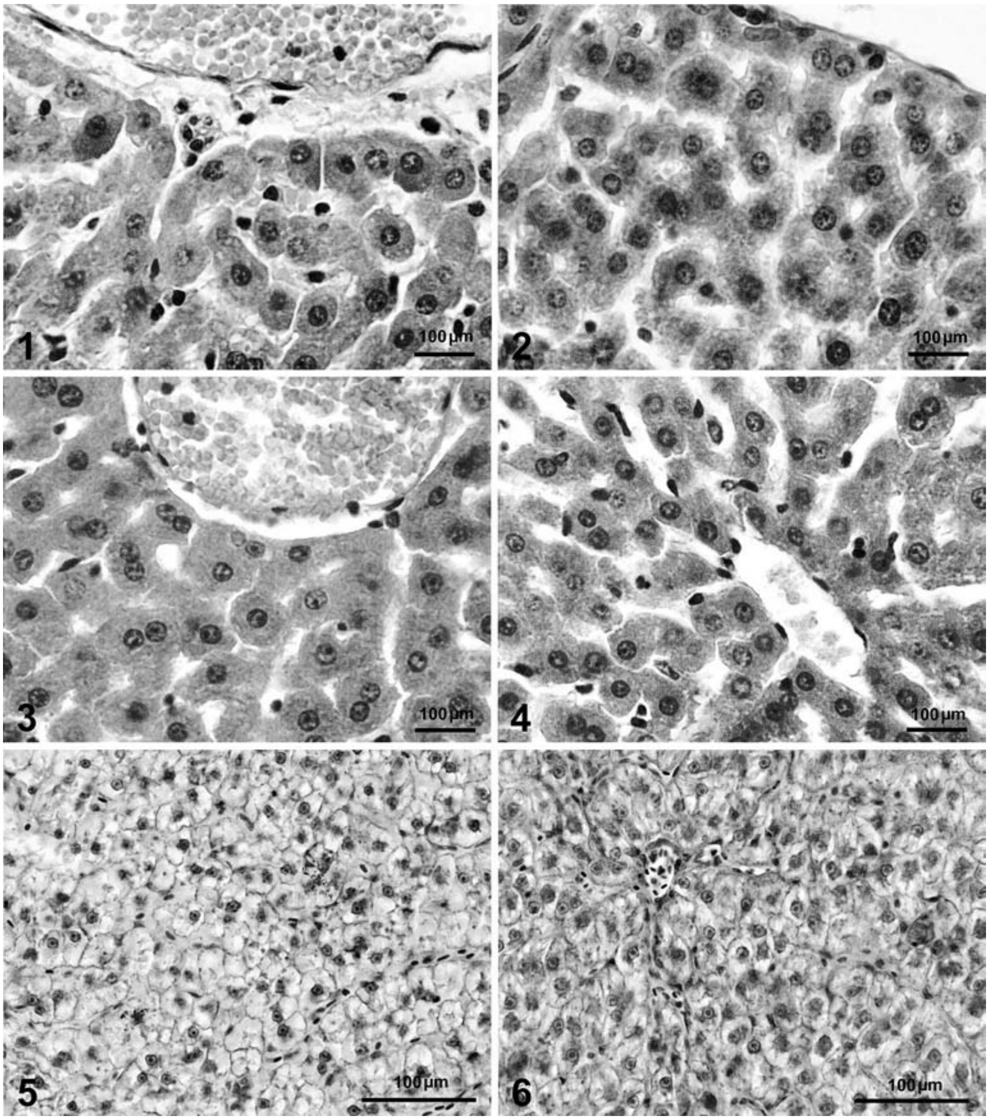

Fig. 1-6. Liver of animals living nearby pesticide dump – microscopical lesions. HE staining

1, 2. Black striped-field mouse (*Apodemus agrarius*, Pallas 1771) – parenchymatous degeneration, hyperaemia (Fig. 1), edematous in the connective tissue under the blood vessel wall (Fig. 1), binucleate hepatocytes. HE staining.

3, 4. Yellow-necked mouse (*Apodemus flavicollis,* Melchior 1834) – parenchymatous degeneration, hyperaemia, binucleate hepatocytes. HE staining.

5, 6. Carp (*Cyprinus carpio* L.) – parenchymatous degeneration, steatosis adipose simplex, melanomagraphages. HE staining.

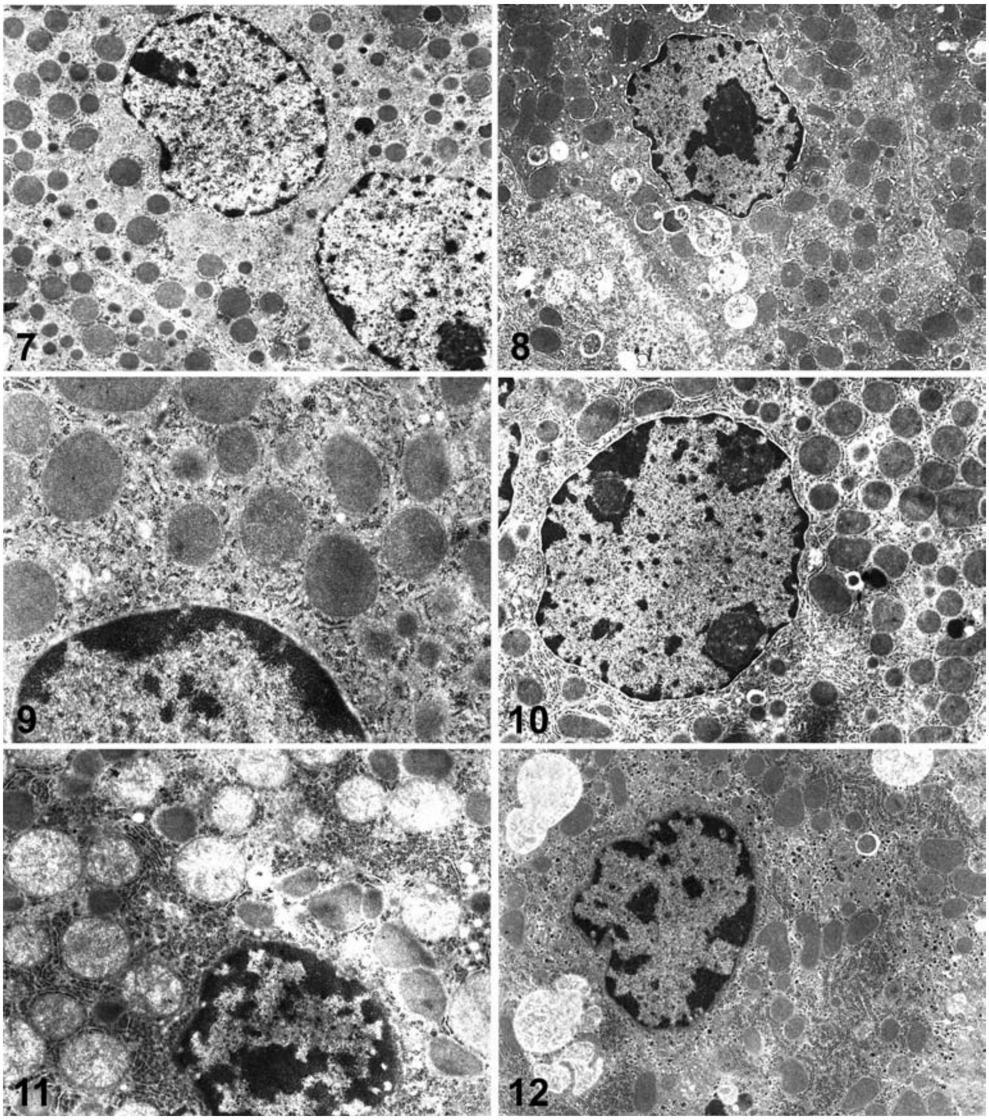

Fig. 7-12. Ultrastructural pattern of the liver mouse living nearby pesticide dump

7. Black striped-field mouse (*Apodemus agrarius*, Pallas 1771) - fragment of binucleate hepatocyte with proliferation of mitochondria. Magn. x 3300.
8. Black striped-field mouse (*Apodemus agrarius*, Pallas 1771) – fragment of hepatocytes: proliferation of mitochondria and edematous few of them, rarefication of cytoplasm (preliminary stage to necrosis). Magn. x 3300.
9. Black striped-field mouse (*Apodemus agrarius*, Pallas 1771) – fragment of hepatocyte with defragmentation of rough endoplasmatic reticulum. Magn. x 16600.
10-12. Yellow-necked mouse (*Apodemus flavicollis,* Melchior 1834) with lesions of mitochondria – proliferation, defragmentation of rough endoplasmatic reticulum (Fig. 10, magn. x 4800), rarefication of matrix in mitochondria (Fig. 11, magn. x 7800), edematous of mitochondria (Fig. 12, magn. x 4800).

10. Summarized

The obtained results lead to the conclusion that the area of the pesticide tomb and surrounding ecosystems was less contaminated with DDT and its derivatives in the period when the disposal site existed. The process of pesticide tomb liquidation resulted in secondary pollution of the environment. Additionally, it was found that mice living in the polluted area accumulated DDT contamination to a degree making it possible to trace changes in its concentration in the environment by analysing the level of those compounds in mouse tissues. The described image of morphological lesions observed in mice reflects contamination of the natural environment the field mice inhabited. Therefore, the mouse liver can be considered a biomarker in the discussed studies, and a morphological examination can be used as a tool in this assessment. A similar relationship was found for the examined carps. Both the location of pathological lesions and their character indicate that their emergence can be attributed to the environment in which the carps lived (Graumann, Drucker 1991, Sawicka-Kapusta *et al.* 1990, Szarek *et al.* 1997, Burton *et al.* 2001, Pawert *et al.* 1998, Schramm *et al.* 1998, Schweiger *et al.* 1997, Szarek *et al.* 2000, Triebskorn *et al.* 1997). Additionally, the fact that adverse pathological lesions in hepatopancreas were much more frequent in fish from the breeding pond situated near the pesticide tomb than in the control carps, carp hepatopancreas (particularly hepatocytes) can be considered a biomarker and it morphological evaluation can be used in this assessment (Pawert *et al.* 1998, Schwaiger *et al.* 1997).

The positive role of biomarkers in a similar perspective was also emphasized by other authors (Depledge, Fossi 1994, Pawert *et al.* 1998, Schramm *et al.* 1998, Schweiger *et al.* 1997).

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