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ASPECTS REGARDING THE MACROSCOPIC AND MICROSCOPIC MORPHOPATHOLOGICAL PICTURE IN CASES OF POISONING IN DIFFERENT ANIMAL SPECIES, WITH SOME PESTICIDES USED IN AGRICULTURE

ASPECTE PRIVIND TABLOUL MORFOPATOLOGIC MACROSCOPIC ȘI MICROSCOPIC ÎN CAZUL INTOXICAȚIILOR LA DIFERITE SPECII DE ANIMALE, CU UNELE PESTICIDE UTILIZATE ÎN AGRICULTURĂ

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ABSTRACT

In order to increase production, modern agriculture uses a multitude of pesticide substances, with the help of which it manages to control crop pests. These substances, unfortunately, remain in the plants on which they are used and can become the main factor of intoxication for certain animals that ingest them both in their pure form and after accumulation in plants. Another, far more serious situation is when substances used as pesticides are used intentionally by humans, such as to poison domestic animals. Among the pesticide substances more frequently used in agriculture and identified in the corpses received in the laboratory with the suspicion of poisoning were endosulfan, terbufos and tebuconazole. The products were identified following the toxicological examination of the gastric contents using the liquid-chromatography technique coupled with mass spectrometry, correlated with the anatomopathological changes identified during the necropsy of the animals and with the histopathological changes observed in the examined organs. The following cases of poisoning were studied: accidental poisoning in eight cases of wild leporids found dead in a field previously treated with the fungicide tebuconazole; intentional poisoning with terbufos in four animals of the canid species; poisoning of twelve goats with endosulfan; and poisoning of five felines with endosulfan.

Keywords: terbufos, tebuconazole, endosulfan, poisoning, intoxication

REZUMAT

Agricultura modernă utilizează în scopul creșterii producțiilor o multitudine de substanțe pesticide cu ajutorul cărora reușește să controleze dăunătorii culturilor. Aceste substanțe, din păcate, au remanență în plantele asupra cărora sunt utilizate și pot ajunge factor principal de intoxicație pentru anumite animale care le ingeră atât sub forma lor pură, cât și în urma acumulării în plante. O altă situație cu mult mai gravă este cea în care substanțele utilizate drept pesticide sunt folosite în mod intenționat de către oameni, cum ar fi pentru otrăvirea animalelor domestice. Dintre substanțele pesticide utilizate mai frecvent în agricultură și identificate în cadavrele recepționate în laborator cu suspiciunea de otrăvire au fost endosulfanul, terbufosul și tebuconazolul. Produsele au fost identificate în urma examenului toxicologic din conținutul gastric prin tehnica de lichidcromatografie cuplată cu spectometrie de masă, corelate cu modificările anatomopatologice identificate cu ocazia necropsiei animalelor și cu modificările histopatologice observate în organele examinate. Au fost studiate următoarele cazuri de intoxicații: intoxicație accidentală la opt cazuri de leporide sălbatice găsite în exitus pe un câmp tratat în prealabil cu fungicidul tebuconazol, otrăvirea intenționată cu terbufos la patru animale din specia canide, intoxicația a douăsprezece caprine cu endosulfan și cu aceeași subtanță otrăvirea a cinci feline.

Cuvinte cheie: terbufos, tebuconazol, endosulfan, otrăvire, intoxicație

The pesticide industry has grown considerably in the second half of the 20th century. During this period, over 30,000 active substances are used in agriculture. 800 of them have been approved in the European Union. Worldwide, the use of pesticides has continued to increase for more than half a century. Their use is increasing in developing countries and decreasing in developed countries (3). It is known that organophosphorus pesticides (e.g., terbufos) are extremely neurotoxic, their main action being the irreversible inhibition of acetylcholinesterase, producing the accumulation of acetylcholine in motor neurones and thus blocking nerve impulses, the death of animals occurring following the blockage of the respiratory nerve centres (vagus nerve nucleus), which induces the blockage of respiratory movements and death by suffocation (4). Terbufos, identified in the gastric contents of four canids, is an organophosphorus insecticide with the molecular formula C9H12O2PS3 and appears as a clear, colourless or slightly yellowish liquid with a strong mercaptan odour. It is soluble in water and was proba-

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bly used for poisoning purposes (1). The most common route of entry into the body is through ingestion (1), but it is also rapidly absorbed through the skin. Following metabolism, the resulting compounds are excreted in the urine and faeces. Other compounds such as endosulfan induce intraneuronal oxidative stress, leading to neuronal damage, and by releasing metal ions from the composition (Cu2+, Zn2+, Mn2+) during biotransformation, they can stimulate oxidation induced by certain reactive oxygen species on lipids and proteins or can inactivate certain ensymes leading to neurotoxic effects (5). Endosulfan is a pesticide in the form of cream-brown crystals with a strong odour and a density higher than water, but almost insoluble in it. The LD50 is 35 mg/kg body weight and was identified in the gastric contents of the five cats found in the exitus with suspicion of poisoning, but also in the case of twelve goats that are supposed to have accidentally ingested plants treated with this insecticide (Table 2). Another pesticide identified in the gastric contents of eight wild rabbit corpses found in the exitus in a field near a forest was tebuconazole. This is a very powerful fungicide, used to prevent the development of fungi in cereal crops, which accumulates in plants and can induce intoxication in animals that eventually feed on the plants in question. Tebuconazole is presented in the form of a colourless or white, odourless powder, soluble in water. Following ingestion, it is eliminated through urine and faeces.

This paper aims to present a case study conducted during the years 2023-2024 on accidental or intentional poisonings with various pesticide substances, the most frequently encountered of which were tebuconazole, terbufos and endosulfan (4). The cases brought for the purpose of establishing the cause of death by laboratory methods carried out within the framework of the forensics were represented by various species of animals, domestic and wild (Fig. 1 and Fig. 2), but the most frequently reported and investigated were the cases of poisoned domestic felines and canines (2).

MATERIALS AND METHODS

Over the two years of study considered, a number of 188 cases were examined in 2023 and 185 cases in 2024 (total 373) (Table 1) of animal abuse or intentional killing without right, cases which, in order to be resolved and to determine the cause of death of the respective animals, were brought by the Animal Protection Office within the county police stations and the municipality of Bucharest, as evidence in criminal cases, to the Institute of Animal Diagnostics and Health with the aim of carrying out the veterinary forensic laboratory expertise and identifying the cause of death. Veterinary forensic laboratory expertise includes all laboratory analyses that are undertaken in order to establish the cause of death, whether suspected or not, as the case may be. Thus, both in the case of the eight samples represented by hares in which the fungicide tebuconazole was identified in the gastric contents, as well as in the case of the five cats and twelve goats intentionally or accidentally poisoned with endosulfan, and in the case of the four dogs intentionally poisoned this time with terbufos, the basis of the laboratory analyses was the necropsy procedure for small and medium-sized animals, followed later by the collection and sampling of samples for the performance of complementary laboratory analyses.

Table 1

Number of samples and animal species received and analysed during 2023-2024 with suspicion of intoxication and confirmation

No.	Species	2023		2024	
		Suspicion	Confirmation	Suspicion	Confirmation
1	Canine	94	64	67	38
2	Feline	58	33	56	26
3	Cattle	0	0	12	12
4	Goats	10	7	8	5
5	Sheep	7	3	13	9
6	Pigs	3	2	0	0
7	Wild hares	0	0	14	8
8	Horses	3	0	0	0
9	Birds	12	6	15	4
10	Jackal	1	1	0	0
	Total	188	116	185	102

Table 2

Animal species confirmed to be poisoned with different types of pesticides in the period 2023-2024

Species	Pesticide	2023	2024	Total
Dogs	Terbufos	3	1	4
Cats	Endosulfan	2	3	5
Goats	Endosulfan	12	0	12
Wild hares	Tebuconazole	0	8	8
Total		17	11	29

As integral parts of the final analysis report, in the case of animals poisoned with pesticides, starting from the suspicion of poisoning, the following laboratory examinations were performed: individual anatomopathological and histological examination for each animal, toxicological examination, but also other additional investigations such as bacteriological examinations and parasitological examination as appropriate, in order to exclude other causes that could have contributed to or determined the death of the animals. Since, in a number of 155 of the 373 cases examined during the years 2023 and 2024, at the end of the expertise the cause of death of the animals in question was different from what was suspected at the beginning of the investigations; at the time of receipt of the samples, complementary laboratory examinations are essential when there is no possibility of certainty (Table 2). Moreover, for the morphopathologist veterinarian who performs veterinary forensic laboratory expertise, the expertise is much more complicated than in the case of human medicine, starting from the fact that the animals investigated do not present, in most cases, a detailed medical history as is the case with humans. For the additional laboratory examinations, representative samples were taken from the following organs: kidneys, liver, lungs, heart, pancreas, central nervous system, gastric contents, unopened long bone and intestinal fragments. These samples were processed separately and simultaneously for several complementary tests that complement each other, namely the toxicological examination through the screening test for pesticide identification by liquid chromatography coupled with mass spectrometry from pathological material and the histological examination by specific staining methods to highlight the histopathological changes that can be attributed to a certain type of toxicant identified.

Methods

For the 188 cases received in 2023 and the 185 cases received in 2024 within the national reference laboratory for veterinary forensic laboratory expertise, in order to identify the toxic compound present in the gastric or liver contents, as the case may be, a necropsy examination was performed in a preliminary phase by specifying the anatomopathological changes. The necropsy examination involves following a standard procedure for performing a necropsy in small and medium-sized mammals, being slightly more complicated in medium-sized animals, in this case in goats, due to the size of the respective animal; therefore, sometimes the procedure may undergo slight changes on site, as follows: general examination of the corpses, examination of the skin and gills, examination of apparent mucous membranes, examination of subcutaneous connective tissue, opening and examination of natural cavities (abdominal cavity, thoracic cavity, cranial cavity), and evisceration and examination of the organs in the cavities. During all stages of examination and sectioning, portions of organs or anatomical parts were taken, namely representative fragments of the liver, kidneys, brain, and lung for histological examination, as well as gastric and liver contents, in the case of corpses in which the necropsy examination suggests an anatomopathological picture specific to poisoning with various toxic substances, for toxicological examination, in this case for the detection of toxic compounds from the pesticide class.

The fragments of organs harvested during the necropsy examination are subjected to the specific stages of the histopathological method of identifying histological and topohistological structures through the histological staining method Haematoxylin-Eosin (H. E.). The histopathological method of examining tissues and organs represents one of the most appreciated and conclusive diagnostic methods since, not infrequently, lesions that could not be visible during macroscopic examination are outlined during microscopic examination, thus rendering the real anatomo-pathological and histopathological picture of the investigated material and, often, even showing the cause of death of the investigated animal.

In order to identify pesticides in the gastric contents of the investigated animals, a physicochemical method called gas chromatography coupled with mass spectrometry was used. The technique called gas chromatography coupled with mass spectrometry (GC /MS) separates chemical mixtures (the GC component) and identifies the components at the molecular level (the MS component). It is one of the most precise tools for analysing environmental samples and beyond. Gas chromatography works on the principle that a mixture will separate into individual substances when heated. The heated gases are transported through a column with an inert gas (such as helium). As the separated substances exit the column opening, they flow into the MS. Mass spectrometry identifies compounds by the mass of the analyte molecule. A "library" of known mass spectra, covering several thousand compounds, is stored on a computer. Mass spectrometry is considered the only definitive analytical detector, which represents scientific evidence in the case of veterinary forensic laboratory expertise.

Following toxicological examinations, pesticides were identified and not quantified through a unit of measurement; therefore, this technique is qualitative and not quantitative, which demonstrates that, once identified, it is toxic to animals and humans and can cause neurological disorders followed by death.

RESULTS AND DISCUSSIONS

Following the anatomo-pathological examinations carried out on a number of 400 cadavers examined between 2022-2024, common aspects of poisoning with different types of pesticides were observed in various species of domestic and wild animals examined, aspects that raised the issue of a differential diagnosis from ethylene glycol poisoning. This suspicion was excluded, however, because certain aspects of the natural environment in which the animals concerned live (such as the hare) excluded this possibility, and in the

case of domestic animals such as dogs or cats that could be susceptible to this type of ethylene glycol poisoning, the histological examination of the kidneys is revealing. On the other hand, the anatomo-pathological picture also differs significantly due to the amount of toxicant ingested and the physiological state of the animals at the time of ingestion (gestation, obesity, etc.). Pesticides used very frequently in agriculture differ in toxicity, but once ingested, they are rapidly absorbed by the digestive mucosa, and their main route of action is at the central nervous system level, and anatomopathological changes determined by the metabolites of these substances can also be observed at the liver and kidney level.

The anatomo-pathological modifications observed following the necropsy examination were slightly different. Thus, during the period under study, eight corpses of hares taken from a field near the forest were examined. A large number of dead animals (approximately 25) were found, and for the veterinary forensic laboratory expertise and determination of the cause of death, eight corpses of different ages and sexes and also in different physiological states were brought. The anatomo-pathological examination was performed on two females and six males, of which one was pregnant and one was non-pregnant, the latter being obese (Fig. 1). In the case of all eight wild leporidae studied, the main anatomo-pathological changes following tebuconazole ingestion were located in the parenchymal organs but also in other tissues. The following were observed: accentuated vascular ectasia in the subcutaneous and intradermal vessels; apparently anaemic mucous membranes; in the cranial cavity, haemorrhagic foci were observed, miliary on the dorsal surface of the cranial skull, haemorrhagic infiltrations in the brain meninges and spinal cord, haemorrhages in small foci in the central nervous tissue, serous or sero-haemorrhagic effusions in the tracheal lumen and in the thoracic and abdominal cavities; inconsistent in the eight animals (three of them showed effusions, and five did not) (Fig. 2). In the case of the pregnant female, haemorrhage was also observed in the uterine horns and congestion and haemorrhage of the products of conception and foetal membranes (Fig. 4). In the case of the eight animals intoxicated with tebuconazole, the main macroscopically visible anatomopathological changes were pulmonary congestion (Fig. 3), haemopericardium and cortico-medullary congestion in the kidney, and inconsistently also being accumulations of haemorrhagic or sero-haemorrhagic fluid in the natural cavities.

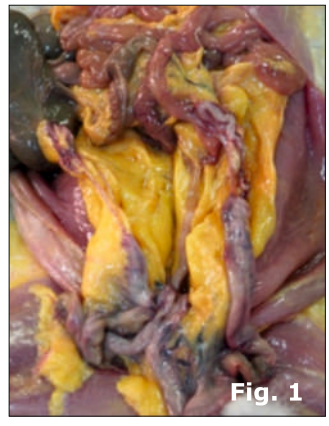

Fig. 1. Hare, female, pregnant, tebuconazole poisoning, very large amount of adipose tissue

Fig. 2. Hare, male, tebuconazole poisoning, hemoperitoneum

During the period under study, seventeen samples representing the corpses of domestic animals, five cats and twelve goats, were received, which presented sudden death following the consumption of toxic substances. Following the veterinary forensic laboratory expertise and the toxicological examination correlated with the anatomopathological examination, the cause of death was identified as poisoning with endosulfan, a powerful insecticide frequently used in agriculture. The results of the anatomopathological examination were slightly different in felines compared to goats, the differences having as their main cause the different anatomo-physiology of the two animal species, some being carnivores and the others herbivores with multicompartment digestive systems and completely

Table 3

Comparative anatomopathological aspects of endosulfan poisoning in felines and goats

Affected organ/tissue	Feline	Goats
Subcutaneous connective tissue	Moderate vascular ectasia	Moderate vascular ectasia
Serous-bloody discharge from the oral cavity and nasal cavities	Present, abundant	Present, abundant
Serous-haemorrhagic effusions in cavities	Present in the thoracic cavity and absent in the abdominal cavity, hyperpericardium	Present in both the thoracic and abdominal cavities, without haemopericardium
Brain	Vascular ectasia	Vascular ectasia and cerebral congestion
Lung	Massive congestion	Emphysema
Kidney	Corticomedullary congestion, pelvic haemorrhage	Kidneys in massive autolysis, no changes
Liver	Congestion	No changes

different feeding methods, being highlighted by anatomopathological changes such as vascular ectasia and cerebral congestion in goats (Fig. 9), and massive pulmonary congestion (Fig. 6) and cortico-medullary congestion and haemorrhage in the renal pelvis in felines (Fig. 8). The comparative anatomopathological aspects are presented in the table below (Table 3).

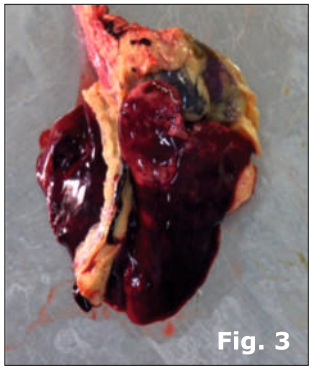

Fig. 3. Hare, female, pregnant, tebuconazole poisoning, severe pulmonary congestion

Fig. 4. Hare, pregnant female, tebuconazole poisoning, uterine and foetal membrane haemorrhage

Another pesticide identified in the gastric contents taken from four dogs received for veterinary forensic laboratory expertise and establishing the cause of their death was terbufos. Terbufos is a pesticide used to increase agricultural production and is frequently identified in households. In these cases, the use of the pesticide was done intentionally, with the aim of poisoning the animals. The anatomopathological picture included the following changes: signs of repeated vomiting as a result of irritation of the gastric mucosa, hyphaemia, massive congestion of the conjunctiva, when opening the cranial cavity, massive haemorrhage and cerebral congestion (Fig. 5), meningeal haemorrhage, haemoperitoneum with blood accumulation in an amount of approximately 2 litres (Fig. 10), haemothorax, haemopericardium, and congestion of parenchymal organs.

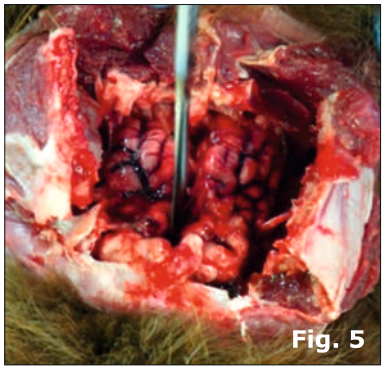

Fig. 5. Dog, male, terbufos poisoning, cerebral and meningeal haemorrhage

Fig. 6. Cat, endosulfan poisoning, severe panlobar pulmonary congestion

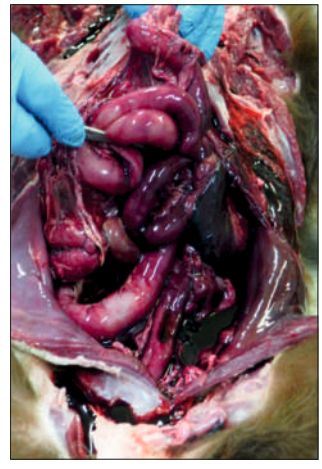

Fig. 7. Dog, male, terbufos poisoning, hemoperitoneum

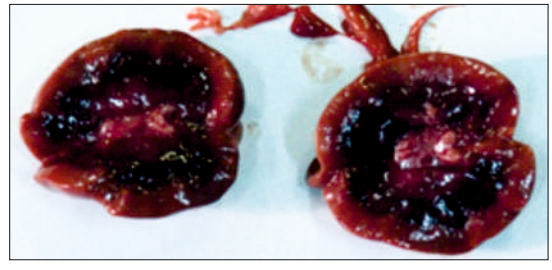

Fig. 8. Cat, male, endosulfan poisoning, cortico-medullary congestion in the kidneys

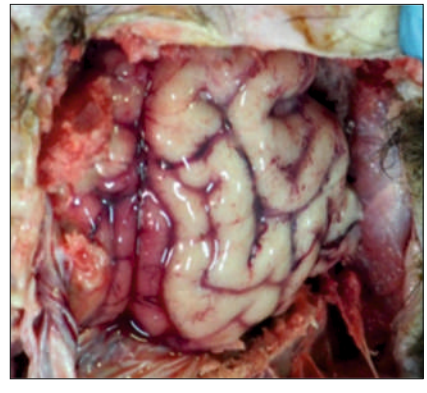

Fig. 9. Goat, endosulfan poisoning, vascular ectasia and cerebral congestion

The histopathological examination of organs and systems revealed aspects frequently encountered in pesticide poisoning in animals. Among the general histopathological changes encountered in the main parenchymal organs of the four animal species studied, we mention: at the heart level, interstitial oedema, interfibrillar haemorrhages and microcenters with hyalinization of cardiomyocytes were observed; at the lung level, obliteration of the alveolar and bronchiolar space by a pronounced haemorrhagic infiltrate, zonal microcenters with hyperaemia and intra-alveolar oedema (Fig. 10, D), thickening of the visceral pleura and necrotic desquamative processes at the level of the bronchiolar epithelium (Fig. 10, C); at the kidney

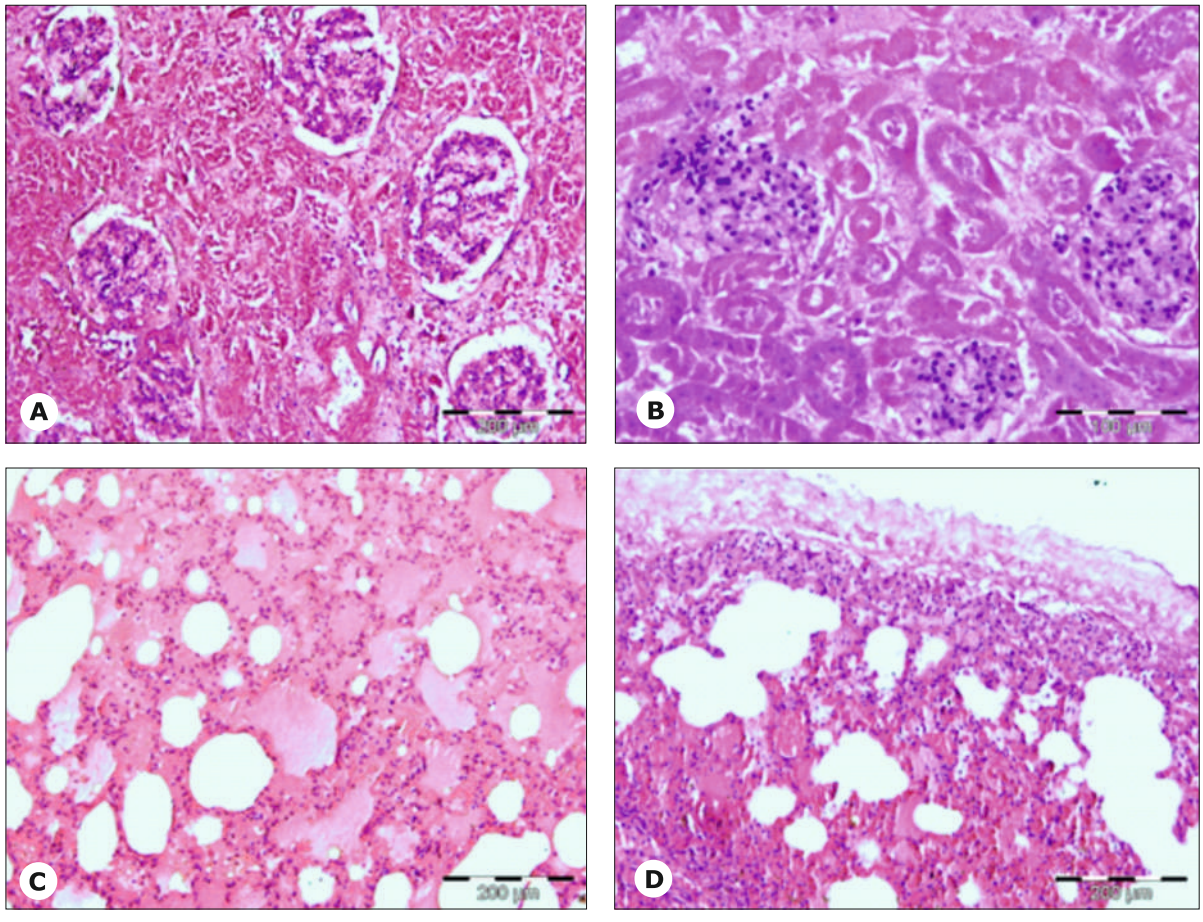

Fig. 10. Microscopic aspects observed after analysing tissue fragments taken from the corpses of animals intoxicated with pesticides: **A** - kidneys, cat intoxicated with endosulfan, HEx100; **B** - kidneys, dog intoxicated with terbufos, HEx100; **C** - lung, goat intoxicated with endosulfan, HEx100; **D** - lung, hare intoxicated with tebuconazole, HEx100

level, massive haemorrhages in the renal cortex, necrosis of the proximal renal tubules in the cortex (Fig. 10, A) with the presence of renal glomeruli in various stages of mesangium disorganization (Fig. 10, B), non-uniform representation of the glomerular filtration space, without inflammatory reaction (acute renal failure following pesticide poisoning). Also, the kidney is the site of a long series of anatomopathological changes following poisoning, such as swelling and disorganisation of the renal tubules in the cortex expressed by thickening and non-uniform undulations of the Bowman's capsule and intense necrobiotic processes in the reno-epithelial cells. In particular, differences could be observed at the microscopic level in terms of the comparative histopathological picture; differences are presented in Table 4.

All histological aspects reinforce the macroscopic picture, that of haemorrhages and haemorrhagic infiltrate at the level of the central organs, which is concluded by specifying the cause of death as certainly that of pesticide poisoning (Fig. 10).

Following the laboratory toxicological examination, the presence of various pesticides (terbufos, tebuconazole, endosulfan) at the level of the gastric contents was identified, through specific techniques, and the results were interpreted by the presence of this compound in the sample to be analysed, which represents a qualitative and not quantitative result. Thus, if even traces of toxic compounds were identified, in the final conclusion of the analysis report, the identified toxic substance, respectively the pesticide, will be stated as the cause of death.

CONCLUSIONS

Given that pesticides are widely used in increasing agricultural productivity and that they are quite easy to obtain by farmers, they either, due to poor information or with bad intentions, use them intensively, and these poisonings are often encountered in animals. From the point of view of the lesions caused by these substances, following ingestion or inhalation in the animal body, regardless of the species, it can be observed that the main organ affected immediately after pesticide ingestion is the gastric mucosa through which it is absorbed into the body (a fact demonstrable by repeated vomiting in species that have this physiological reflex as a method of protection, but also by gastritis observed after necropsy and haemorrhages in the gastric submucosa), and then, after metabo-

Table 4

Comparative histopathological aspects in the pesticide poisonings studied

Affected tissue	Endosulfan	Terbufos	Tebuconazole
Pulmonary tissue	active hyperaemia, alternating with areas of non-inflammatory intraalveolar oedema and interstitial haemorrhagic infiltrates; bronchiectasis with flattening of the bronchiolar epithelium	obliteration of the alveolar space by a pronounced haemorrhagic infiltrate, zonal microcentres with hyperaemia and intra-alveolar oedema	pronounced intra-alveolar oedema, with areas of obliteration of alveolar architecture; interstitial haemorrhagic infiltrates and active intraseptal hyperaemia
Kidney tissue	cortico-medullary interstitial haemorrhagic infiltrates; hyperaemia in the glomerular mesangial capillaries.	haemorrhages in the renal structure, ectasia of the veins of the renal portal system as well as haemorrhages in the cortico-medullary	hyperaemia and cortico-medullary haemorrhages, alternating with vacuolar degeneration in the convoluted tubule renoepithelial cells; haematic casts in the medulla
Liver tissue	oedema in the spaces of Disse accompanied by hyperaemia and microhaemorrhages; hepatocyte granulo-vacuolar degeneration; zonal hemosiderosis	haemorrhagic infiltrates, severe granulo-vacuolar degeneration with disappearance of cell boundaries	pronounced interstitial oedema; interstitial haemorrhagic microfoci; panlobular hepatocyte granulo-vacuolar degeneration accompanied by intense necrobiotic processes and zonal hepatocytolysis
Central nervous tissue	microfoci of necrosis in the neuropil with diffuse perivascular haemorrhagic infiltrates; hyperaemia due to vascular ectasia; low-intensity zonal gliosis; nonspecific vacuolisation in the neuropil	Diffuse perivascular haemorrhagic infiltrates	hyperaemia due to vascular ectasia; low-intensity zonal gliosis

lism, organs such as the central nervous system, lungs and kidneys are affected. The variations in the intensity of the lesions are due to multiple factors related to the species (in goats, an increased intensity of congestive lesions is observed compared to felines in the case of endosulfan poisoning), age (lesions characterized by massive haemorrhage with more severe vascular ectasia were observed in young animals), physiological state (pregnant females presented congestion and haemorrhage of the pregnant uterus as well as of the foetuses and foetal membranes as well as massive congestion in the lungs compared to males or females that were not pregnant) and the method of entry of the toxicant into the body (the toxin entered by ingestion acts more slowly and a larger amount of substance is required to produce lesions incompatible with life, compared to the inhaled toxin that acts quickly and produces massive congestive lesions in the lungs and central nervous system). A greater intensity of anatomopathological changes such as congestion and haemorrhage was observed in canids, probably related to the feeding mode of this species (rapid ingestion, sometimes without the necessary mastication). In summary, it can be stated that the macroscopic and microscopic anatomopathological changes identified in the poisonings with the pesticides studied (endosulfan, terbufos, tebuconazole) target congestion and haemorrhage at the level of parenchymal organs, especially the vital ones (brain, lung), and in the case of terbufos, which acts directly on acetylcholine at the level of motor neurones, the death of the intoxicated animals is due to the blockage of the transmission of nerve impulses and cardio-respiratory arrest.

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