UNIVERSITY OF AGRICULTURAL SCIENCE AND VETERINARY MEDICINE OF THE BANAT COUNTY TIMISOARA
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ABSTRACT

of the doctoral thesis:

INFLUENCE OF ANTHROPOGENIC POLLUTANTS ON DOLPHIN CONSERVATION IN THE ROMANIAN BLACK SEA COASTAL WATERS

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Key words: heavy metals, organochlorine compounds, polycyclic aromatic hydrocarbons, dolphins, health, The Black Sea

This doctoral thesis has 259 pages, 96 tables, 103 graphs, and 117 pictures, out of which 97 are totally original, and 309 bibliographic titles. This thesis is structured in two parts: Bibliographical study, and Personal contributions.

PART I
BIBLIOGRAPHICAL STUDY

It contains 3 chapters extended on 53 pages.

Chapter 1. ***DOLPHINS*** is divided into two sub-chapters: Taxonomy of the three dolphin species living in the Black Sea, and Morpho-physiology, where the anatomy and physiology of each apparatus and system are described, with emphasis on dolphin peculiarities.

Chapter 2. ***PRESENTATION OF THE MAIN POLLUTANT GROUPS*** is divided into three sub-chapters: Heavy metals, Organochlorine compounds, and Aromatic polycyclic hydrocarbons. Each of these sub-chapters contains the description of the main compounds of the analyzed pollutant group, their presence in the water eco-system, toxicity, toxic and eco-toxic effects.

Chapter 3. ***THE EFFECTS OF POLLUTANTS ON DOLPHINS*** is divided into three sub-chapters, each underlying the effects of a different pollutant category (heavy metals, organochlorine compounds, and polycyclic aromatic hydrocarbons).

PART II
PERSONAL CONTRIBUTIONS

It comprises 4 chapters, extended on 206 pages, containing 91 tables, 103 graphs, 90 original pictures.

Chapter 4. ***THE REASON, AIM AND OBJECTIVES OF THE RESEARCH***

The reason: the presence of pollutant industry on the Black Sea coastline, the little information on pollution of the Romanian Black Sea coastal waters, as well as the limited data on the pollutants the dolphins are exposed to. Another reason was the scarce research on the toxic impact of pollutants on the good health of dolphins.The study of the correlation between the quality/health of the environment and quality of life/health is among the main objectives of national and international research.

The aim: the influence of pollutants of anthropic nature (heavy metals, organochlorine compounds and polycyclic aromatic hydrocarbons) on conservation of the dolphins in the Romanian Black Sea coastal water, and establishing the toxic impact of the above mentioned pollutants on the food cycle sediment-water-fish-dolphin.

Main objectives: evaluation of heavy metal pollution (sediment, water, fish and dolphin organs and tissues), evaluation of organochlorine compound pollution (sediment, water, fish and determining the concentration of organochlorine compounds in dolphin blubber), evaluation of aromatic polycyclic hydrocarbon pollutants (sediment, water, fish and determining the concentration of pollutants in the liver of dolphins), establishing pollutant dynamics (heavy metals, organochlorine compounds and aromatic polycyclic hydrocarbons) along the food web sediment-water-fish-dolphin, evaluation of the impact of the studied pollutants on dolphin health (through toxicological and histological tests), and evaluation of chromium toxicity on dolphin cell cultures (determination of chromium citotoxicity on primary dolphin fibroblasts, determination of chromium genotoxicity on dolphin primary fibroblasts, and intra-cellular chromium uptake).

Chapter 5. *MATERIALS AND METHODS*

Sub-chapter 5.1. *Collecting samples*

In order to evaluate the impact of anthropic pollutants (heavy metals, organochlorine compounds and aromatic polycyclic hydrocarbons) on dolphin conservation status, and to establish the toxic impact of the above mentioned pollutants on the food cycle sediment-water-fish-dolphin we have collected sediment, sea water, fish (*Sprattus sprattus*), and dolphins tissues and organs samples (*Phocaena phocaena*, *Tursiops truncatus* şi *Delphinus delphis*).

From what we have collected we have measured the level of main groups of pollutants: heavy metals (aluminium,cadmium, chromium, copper, iron, manganese,nickel, lead and zinc), organochlorine compounds (DDT, αHCH, βHCH, γHCH and PCB_s), and polycyclic aromatic hydrocarbons (fenanthrene, fluorantheen, anthracene, pyrene, naphtalene, acenaphtilene, acenaphtene, fluorene, crysene, benzo[a]anthracene).

For heavy metals determinations/measurements have been made using the atomic absorbing spectrophotometric method (the Shimadzu AA-6650 spectrophotometer in the Toxicology and Nutrition lab of the Faculty of Veterinary medicine, Timişoara)

The organochlorine compounds have been measured through the chromatographic gas method with electron capture using the Varian 3800 device, in the Regional lab of waste control, at Constanţa DSVSA.

The polycyclic aromatic hydrocarbons have been determined through the chromatographic gas method using a mass spectrometer detector (Clarus 500 device) in the Oceanography lab of the G.A. National Institute of Marine Research, Constanţa.

The data have been statistically interpreted with the help of some non- parametric tests. They have been the Kruskal-Wallis tests (to compare three groups), and the Mann-Whitney tests (to compare two groups).In the case of the non-parametric tests the used parameter was the median. To calculate the arithmetic average, the standard error, the standard deviation, and the 95% confidence level we have used descriptive statistics.

Sub-chapter 5.2. *Evaluation of the impact of pollutants on dolphin health*

The dolphins found dead on the shore, which were not totally decayed, have been studied, mentioning the macroscopic modifications in Necropsy files. We have also taken samples for the histological test. They were kept in alcohol (80) before the histological preparations were made.

The structural modifications of different organs (liver, kidneys, lungs, spleen, heart, ovary, testicle) have been noticed using the optic Olympus CX41 microscope on histologic sections after they were stabilized in alcohol, then dehidrated, paraffine covered and sectioned at 5 μ, *HEA* and *Mallory* tricomic colloration.

Sub-chapter 5.3. *Evaluation of chromium toxicity on dolphin cell cultures*

The study has been conducted in the Wise Laboratory of Environment toxicology of Southern Maine University, portland, Maine, USA.

The stages of this study have been: gathering dolphin lung and skin primary cell lines, followed by conducting experiments of cititoxicity, genotoxicity and intra-cellular chromium uptake.

In this study we have used primary cell lines from the lung and skin of a *Tursiops truncatus* dolphin.The tissue fragments were placed in L-15 containers in penicillin, streptomicine and gentamicine, then transported , at a teperature of 4 degrees Celsius, to Wise lab. The tissue fragments were washed repeatedly with various antibiotics and PBS. Then, the tissue was cut in very small tiny pieces with a sterile scalpel, repeatedly rinsed and then placed in flat T-25 containers in the presence of Dulbecco's Minimal Essential Medium with Ham's F-12 (DMEM/F12) suplimented with 15 precent Cosmic Calf Serum - CCS, 2mM/l glutamin, 100U/ml penicillin/100μg/ml streptomicine and 0,1mM sodium piruvat.

The containers were then placed in incubators with 95% humidity and 5% CO2, at 37 ºC. In each incubator it was a thermometer to allow permanent temperature monitoring. The cells develop on the bottom of the container in one layer, and when they are almost confluent they are re-cultivated.

The cells were kept adherent, sub-confluent in a single layer by feeding them every other day, re-cultivating them at least once a week, then placed in larger containers.

In view of re-culturing the containers were examined with the help of a microscope, placed in laminar hood, the cells were removed from the bottom of the container, placed in a cooling centrifuge, re-suspended in a fresh medium and counted in a cell counter, then re-cultivated in a fresh culture medium.

All experiments were made in the logarithm cell growing mode.

The substance used in the experiments was sodium bichromate, as soluble form.

The experiments were made on *Tursiops truncatus* skin and lung fibroblasts.

Citotoxicity was determined through the chlonogenic method, which measures cell plating efficiency in the groups under experiment compared to the control group, based on the method described by Wise &col, but operating some alterations. The treatment time of the experiments was of 24 and 120 hours.

Genotoxicity evaluated chromosome and metaphase modifications. The chromosome injuries and their evaluation have been described by Wise&col. The treatment time of the experiments was of 24 and 120 hours.

Intra-cellular chromium ions uptake was determined according to the method described by Wise &col. The treatment time of the experiments was of 24 and 120 hours.

Chapter 6. RESULTS, DISCUSSIONS AND CONCLUSIONS

Sub-chapter 6.1. *Presentation of the region – The Black Sea ecosystem*

This sub-chapter comprises the description of the Black Sea, namely: geography, hydrology, chemical properties, sea currents, climate, bio-diversity (characteristics that make the Black Sea a "Unicum hidrobiologicum"), as well as the dolphin food web in the Black Sea.

Sub-chapter 6.2. *Main pollution sources in the Black Sea coastal waters*

In the coastal waters the pollutants are important companies that use and dispose of extremely large quantities of water that have high concentrations of quality indicators.

The main pollutants companies are: Rompetrol Rafinare, Regia Autonomă Judeteană de Apă Constanţa, S.C. Oil Terminal S.A. Constanţa, S.C. Sursal S.A. Saligny, S.C. Lafarge-Romcim S.A. Sucursala Medgidia, S.C. Etermed S.A. Medgidia, S.C. Edilmed S.A. Medgidia, S.C. Legmas S.A. Năvodari, S.C. Edil Prest Babadag, S.C. Pigcom Satu Nou, Midia Harbour, Constanţa Harbour and 2 Mai Shipyard.

Sub-chapter 6.3. *Evaluation of heavy metals pollution*

An evaluation of heavy metal pollution of the sea sediment, sea water, fish and of the dolphin tissue and organs was made.

There have been found exceeding the legal maximal limits (as stipulated in Order No. 161/16.02.2006) in sediment only for the average cadmium level, in water for cadmium, iron, and zinc, while in fish cadmium and lead.

Exceeding the risk limits for the sea creatures that live in the sediment: of cadmium, chromium, and of copper, while in water of cadmium, copper, nickel, and lead.

For dolphins the hepatic levels of cadmium, copper and zinc were within the average reference limits, while for iron and lead the limits were exceeded. The renal levels of cadmium , lead and zinc were within the limits.

Sub-chapter 6.4. *Evaluation of organochlorine compounds pollution of the sea ecosystem*

An evaluation of the pollution of the sea sediment, sea water, fish, and of the dolphin blubber is comprised in this sub-chapter.

There have been found exceeding of the legal maximal limits, as stipulated by Order No. 161/16.02.2006, for γHCH in sediment, and for ΣDDT, γHCH, Σ HCH in water.

Exceeding the risk levels in water, as proposed by British quality standards, of the average concentrations of ΣDDT and ΣHCH, and the risk levels of ΣDDT for wild animals that eat fish.

For dolphins the average levels of organochlorine compounds was in the same range as found in dolphins from other seas and oceans.

Sub-chapter 6.5. *Evaluation of polycyclic aromatic hydrocarbons pollution*

This sub-chapter studies the pollution of the sea sediment, sea water, fish and of the presence of the polycyclic aromatic hydrocarbons in the dolphin liver.

There were found exceeding the legal maximal limits, as stipulated by Order No. 161/16.02.2006, of ΣPAH in sediment, of fenanthrene, anthracene, fluoranthene and, benzo[a]anthracene in water.

Concentrations presenting potential risk for the sea biota of acenaphtene, fluorene, fenanthrene and anthracene in sediment.

For dolphins the average levels of polycyclic aromatic hydrocarbons was higher than found in dolphins in 1990 but in the same range as found in 2000.

Sub-chapter 6.6. *Evaluation of the pollution dynamics along the food web sediment-water-fish-dolphin*

Three things are present here: the pollution dynamics along the food web, the calculation of bio-concentration and bio-accumulation factors, and the interpretation of the data.

The results showed that the sediment is the main reservoir for all studied pollutants. For metals (with the exception of manganese, nickel and zinc) and organochlorine compounds it was seen an increase in concentrations from one level to the other in the trophic web water-fish-dolphin, while for polycyclic aromatic hydrocarbons the dynamic along the trophic web water-fish-dolphin was fortuitous according to the compound.

All studied pollutants have been bioconcentrated in fish, but bioaccumulation in dolphins tissues and organs was according to the compound.

Sub-chapter 6.7. *Evaluation of health status*

The evaluation of the health of the dolphins was made through necropsy and histological examinations.

The anatomo-pathological alterations from liver, kidney, gonads were: congestive and degenerative, proof that the dolphins live in a stressful environment, presenting possible repercussions on their health, reproduction and, subsequently, conservation.

Sub-chapter 6.8. *Effects of chromium on the dolphin fibroblasts*.

The stages of the study were: conduct cell toxicity experiments, conduct genotoxicity experiments, and experiments on the chromium intracellular uptake.

Sodium bicromate was citotoxic and genotoxic for the fibroblast lines from the skin and lungs depending on the tissue and concentration, in direct correlation with the dosage and exposure duration. Intracellular chromium uptake was in direct correlation with the dosage and exposure duration.

The results have been interpreted and critically compared with the scientific data available.

Chapter 7. GENERAL CONCLUSIONS

The research on “The influence of pollutants of anthropogenic nature on dolphin conservation in the Romanian Black Sea coastal waters” has underlined the following:

- **Pollution Dynamics**
  - ***Sediment***
    - *Heavy metals*
      - the sediment is where heavy metals are present in the sea ecosystem;
      - annual variations of heavy metals level, with a maximum, in 2005, for chromium, copper, manganese and zinc, in 2006 for aluminium, nickel and lead, in 2007 for cadmium and iron, and in 2008 for none of the metals under study;
      - different dynamics in 2008 compared to the year 2005: an obvious decrease in iron, copper, manganese, chromium, lead, a limited increase in aluminium, cadmium, and the same levels of nickel and zinc;
      - exceeding the legal maximal limits (as stipulated in Order No. 161/16.02.2006) only for the average cadmium level;
      - exceeding the risk limits for the sea creatures that live in the sediment: of cadmium, for the entire period under study, of chromium, in 2005, and of copper, in 2005 and 2006.
    - *Organochlorine compounds*
      - annual variations of these compounds, with a maximum for all of them in the year 2005;
      - descending dynamics in 2008 compared to 2005 for all the compounds under study;
      - exceeding the legal maximal limits, as stipulated by Order No. 161/16.02.2006, only for γHCH (NB. There are no legal stipulations for άHCH and βHCH).
      - maintaining ΣDDT and ΣPCB concentrations under the risk level for the sea biota.
    - *Polycyclic aromatic hydrocarbons*
      - annual variations of the levels of these hydrocarbons, with a maximum in 2005 of all of the ones studied;
      - alternating dynamics between 2005 and 2008, with a reduction of the concentrations in 2008 compared to 2005 of: fenanthrene, fluoranthene, anthracene, pyrene, naphtalene, acenaphtilene, acenaphtene, fluorene, crysene, benzo[a]anthracene;
      - exceeding the legal maximal limits of ΣPAH, as stipulated by Order No. 161/16.02.2006;
      - concentrations presenting potential risk for the sea biota of acenaphtene, fluorene, fenanthrene and anthracene.

  - ***Sea water***
    - *Heavy metals*
      - annual variations of the heavy metals concentrations, with a maximum in the year 2005 for chromium, copper and lead, for iron in 2006, for cadmium, manganese and nickel in 2007, and for aluminium and zinc in 2008;
      - in the year 2008 as compared with 2005: an increase in the concentrations of manganese, aluminium, iron, zinc and nickel, and a decrease in the concentrations of copper, chromium, cadmium and lead;
      - exceeding the legal maximal limits, as stipulated by Order No. 161/16.02.2006, of the average levels of iron, cadmium and zinc during the period under study (NB. There are no legal maximal limits in the sea water for aluminium and manganese);
      - exceeding the protection level for the sea biota, in the year 2005, of zinc, and cadmium (for fish only), in 2007 of cadmium and nickel, and in 2008 of zinc and copper (NB. There is no stipulated limit for manganese).

- ✓ *Organochlorine compounds*
  - annual variations of the levels of these compounds, with a maximum in 2005 for ΣDDT, γHCH and ΣPCB, and in 2006 for άHCH and βHCH;
  - different dynamics in the year 2008 compared to the year 2005: a decrease of the ΣPCB, γHCH, ΣDDT levels; an increase of άHCH, and the same level of βHCH;
  - exceeding the legal maximal limits, as stipulated by Order No. 161/16.02.2006, of ΣDDT, γHCH, Σ HCH (NB. Legal maximal concentrations of άHCH, βHCH şi ΣPCB are not published),
  - exceeding the risk levels, as proposed by British quality standards, of the average concentrations of ΣDDT and ΣHCH, and exceeding the risk levels of ΣDDT for wild animals that eat fish.
- ✓ *Polycyclic aromatic hydrocarbons*
  - annual variations of the levels of these hydrocarbons, with a maximum in 2005 for all these substances under study;
  - random dynamics between 2005 and 2008, with a concentration reduction in the year 2008 of all hydrocarbons under analysis;
  - exceeding the legal maximal limits, as stipulated by Order No. 161/16.02.2006, with the exception of naphthalene (NB. There are no legal stipulations for acenaphtilene, acenaphtene, fluorene, pyrene and crysene).

➢ ***Fish***

- ✓ *Heavy metals*
  - annual variations of their concentrations, with a maximum in 2005 for nickel and zinc, in 2006 for iron, and in 2007 for aluminium, cadmium, chromium, copper, manganese and lead;
  - in 2008, compared to 2005: an increase of the level of manganese, and aluminium, and a decrease of iron, nickel, lead, cadmium, chromium, zinc and copper;
  - exceeding the legal maximal limits, as stipulated by the European Commission Regulation No. 1881/19.12.2006, of lead and cadmium (NB. There are no legal maximal limits for aluminium, chromium, copper, iron, manganese or zinc.).
- ✓ *Organochlorine compounds*
  - annual variations of the levels of these compounds, with a maximum in the year 2005 for ΣDDT, βHCH and ΣPCB, in 2006 for άHCH, and in 2008 for γHCH;
  - descending dynamics between 2005 and 2008 for all these compounds;
  - ΣPCB did not exceed the legal limits, as stipulated by the European Commission Regulation No. 1881/19.12.2006 (NB. There are no limit stipulations for the other compounds);
  - exceeding the risk levels of ΣDDT for wild animals that feed on fish, as stipulated by the Canadian Agency for the protection of the environment (NB. There are no stipulations for the other compounds).
- ✓ *Polycyclic aromatic hydrocarbons*
  - annual variations of the levels of these substances, with a maximum in 2005 for all of them, except for naphtalene, benzo[a]anthracene in 2006 and pyrene in 2007;
  - lower concentrations in 2008 than in 2005 of the polycyclic aromatic hydrocarbons under study, except for naphtalene.

➢ ***Dolphins***

- ✓ *Heavy metals*
  - different accumulation, according to the metal and tissue/organ, the order being as follows: *cadmium*: kidneys, liver, lungs, sexual organs, muscular tissue, heart and brain; *copper*: liver, heart, kidneys, lungs, brain, muscular tissue, sexual organs and cartilage; *lead*: liver, cartilage, sexual organs, kidneys, brain, lungs, heart and muscular tissue; *zinc*: cartilage, liver, heart, lungs, muscular tissue, sexual organs, kidneys and brain;

- as for the liver, the levels of cadmium, copper and zinc were within the average reference limits, while for iron and lead the limits were exceeded, and for manganese the level was below the limit (N.B. For aluminium, chromium and nickel there have no been found references);
- as for the kidneys, the levels of cadmium , lead and zinc were within the limits, while those of copper were below the limit (N.B. For aluminium, chromium, iron, manganese and nickel there have no been found references);
- the influence of the species($p>0.05$) on the level of liver accumulation: *Phocoena phocoena* presented the highest concentration for aluminium, chromium and nickel, *Tursiops truncatus* of copper and iron, and *Delphinus delphis* of cadmium, manganese, lead and zinc;
- the sex influence ($p>0.05$) on the liver accumulation of the three species of dolphins living in the Black Sea: the females accumulated larger concentrations of cadmium, iron, lead and zinc, while the males: aluminium, chromium, copper and manganese;
- intra-specific sex differences as for the liver accumulation levels ($p>0.05$): the *Phocoena phocoena* males accumulated higher concentrations of aluminium, cadmium, chromium,copper, iron, manganese, lead and zinc than the females, while the *Tursiops truncatus* males accumulated higher concentrations of cadmium, chromium, copper, manganese and lead than the females; the females of both species accumulated larger concentrations of nickel than the males;
- inter-specific female difference: *Phocoena phocoena* accumulated larger liver concentrations of chromium and nickel, *Tursiops truncatus* – aluminium, copper and iron, and *Delphinus delphis* – cadmium, manganese, lead and zinc.

✓ *Organochlorine compounds*

- different accumulation of these compounds in the blubber of the dolphins: ΣDDT > ΣHCH > ΣPCB; out of the HCH izomers and PCB congeners larger quantities of βHCH and PCB153 were found;
- the significant species influence for αHCH and βHCH (*Phocoena phocoena*> *Tursiops truncatus*), and insignificant as for ΣDDT, γ HCH and ΣPCB (ΣDDT: *Tursiops truncatus*> *Delphinus delphis*> *Phocoena phocoena*, γ HCH: *Phocoena phocoena*> *Tursiops truncatus*> *Delphinus delphis*, while for ΣPCB: *Tursiops truncatus*> *Phocoena phocoena*> *Delphinus delphis*);
- the sex influence ($p>0.05$) on the accumulation level: larger concentrations of βHCH, Endrin, PCB52, PCB138, PCB153 and ΣPCB in the males, while the females presented larger concentrations of ΣDDT, αHCH, γHCH şi PCB180.

✓ *Aromatic polycyclic hydrocarbons*

- the species influence on the liver level of accumulation: *Delphinus delphis* accumulated mainly acenaphtilene, acenaphtene, fluorene, anthracene, fluoranthene, pyrene, benzo[a]anthracene and crysene, while *Phocoena phocoena* – larger concentrations of naphtalene and fenanthrene;
- sex differences as for the liver accumulation level: the females had larger concentrations of acenaphtilene, acenaphtene, fluorene, fluoranthene, benzo[a]anthracene and crysene, while the males accumulated larger quantities of naphtalene, phenantrene, anthracene and pyrene.

❖ **Bio-concentration and bio-accumulation factors**

➢ ***Bio-concentration factors***

- bio-concentration of all the heavy metals studied in the fish (copper>zinc>lead>iron>nickel>chromium>manganese>aluminium>cadmium);
- bio-concentration of all the organochlorine compounds studied in the fish (ΣDDT> ΣPCB> άHCH > βHCH >γHCH);
- bio-concentration of aromatic polycyclic aromatic hydrocarbons in the fish crysene>benzo[a]anthracene>pyrene>fluoranthene>acenaphtilene>naphtilene>acenaphtene>fenanthrene> anthracene, with the exception of fluorene.

- ***Bio-accumulation factors***
  - heavy metals differenciated bio-accumulation according to the tissue or organ (*cadmium*: kidneys; *copper*: cartilage>liver>heart>kidneys>lungs>brain; *lead*: liver; *zinc*: cartilage);
  - differenciated liver bio-accumulation: iron>chromium>aluminium>copper>lead, while manganese, nickel and zinc had a subunitay bio-accumulation factor;
  - different bio-accumulation of organochlorine compounds in the blubber of the dolphins (βHCH > ΣDDT> ΣPCB> άHCH >γHCH);
  - hepatic bio-accumulation of only naphtalene, benzo[a]anthracene, crysene and fenanthrene.

❖ Health status

- the anatomo-pathological alterations were of two types: congestive and degenerative:
  - liver: congestion, peri-vascular edema, intra-arterial thrombosis, intra-vascular endothelial growing, steatosis, hepatic laminae disociation, necrosis/necrobiosis, conjunctive neogenesis and fibrosis;
  - kidneys: passive congestion and degeneration of epithelium (descuamativ nephritis);
  - pulmoni: congestie pulmonară, edem pulmonar, emfizem pulmonar şi bronhopneumonie catarală;
  - lungs: congestion, edema, lung emphysema and catharal bronchopneumony.
  - genitals: ovary injuries (degeneration of folicular epithelium) and testicle injuries (interstitial edema, epithelial degeneration and necrosis);
- the structural alterations in the liver, kidneys and gonads are a proof that the dolphins live in a stressful environment, presenting possible repercussions on their health, reproduction and, subsequently, conservation.

❖ The effects of chromium on dolphin fibroblasts

- the citotoxicity of chromium bicromate for the fibroblast lines from the skin and lungs depending on the tissue and concentration: higher toxicity of those from the skin compared with those from the lungs; toxicity in direct correlation with the dosage and exposure duration;
- potential genotoxicity of this substance for the lung fibroblasts, in direct relatioship with the dosage abd exposure duration;
- chromosome injuries, mainly of the chromatid;
- metaphase modifications, predominantly aneuploidy;
- intracellular chromium uptake in direct correlation with the dosage and exposure duration.

N.B.

An extremely important aspect, that has to be mentioned, is the fact that nationally there is no actual or functional network for monitoring the death of sea mammals in the Black Sea; also, the necropsy of the dead bodies or probing the organs or tissues are not performed.

The few researcherswho are concerned with the sea mammals encounter terrible obstacles in getting biological material and other biological or ecological data; the research on these mammals is not, right now, a priority for Romania. Therefore, the information is not always exhaustive, and result interpretation is difficult, often extremely difficult.