



## Retrospective data analysis of animal poisoning events in Liguria

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### ABSTRACT

Intentional poisoning represents a serious risk to domestic and wild animals, and it can be an environmental and human health issue as well. This paper is a retrospective study, which covers a decade, based on animal poisoning cases and poisoned baits that were submitted for diagnostic examinations to the Veterinary Medical Research Institute for Piedmont, Liguria and the Aosta Valley (IZS-PLVA) in Liguria region. All data were collected through a passive surveillance system introduced in Italy by a decree of the Ministry of Health in January 2009.

43.2% of the animal poisoning cases were confirmed by toxicological analysis, whereas toxic agents were detected in 31.1% of the baits. The most affected animal species were dogs and cats, followed by synanthropic birds. Only 4% of the total poisoning events analysed involved wild animals and cases of livestock poisoning were minimal. An increased number of cases in January, March, April and August was noticed, but no seasonal trend was detected. The most affected areas were the ones with the highest level of urbanization and population density. The major cause of the poisonings and the most common substances detected in the examined baits were anticoagulants whereas cholinesterase inhibitors, organochlorine pesticides and carbamates were detected in a minor number of cases.

This study raises concerns about deliberate animal poisoning in ligurian region and highlights the necessity to fight this phenomenon as it endangers animals, humans and environment.

### 1. Introduction

The widespread phenomenon of intentional animal poisoning can cause severe damage on the environment and animals, including high-value species such as wolves.

The main reason for poisoning are intolerance towards stray or pets, urban hygiene measures, noise and birds chirping; in addition, poison is used to protect human activities such as farming or hunting.

In Europe (Berny et al., 2010; Guitart et al., 2010), and in Italy in particular (Bille et al., 2016) deliberate animal poisoning involves ethical implications and represents a public health danger.

Currently, in Italy, some toxicants are forbidden, but many others are still commercially available. In order to regulate their use and to prevent the potential risks both for human and non-target animals, in 2009 the Italian Ministry of Health issued an ordinance specifying the "Rules on the prohibition of use and detention of baits or poisoned bites". This ordinance states that only specific companies can use baits and authorised biocide products in public areas (Reg. CE 2012/528)

avoiding risk for non-target animals and humans. The ordinance is still in force and forbids the use of poisoned baits and establishes that any suspect animal poisoning or poisoned bait must be reported to competent authorities. Moreover, to detect crimes against animals, anatomopathological and toxicological investigations must be performed for free by Veterinary Medical Research Institutes (IIZZSS) whenever a clinical suspicion is evident. The laboratories are designated as the local reference for the monitoring and toxicological analysis in collaboration with judicial authorities.

However, since 2009, many papers have documented the use of poisoned baits despite the ban. Animal poisoning using poisoned baits has been well documented in many Italian regions (Amorena et al., 2009; Bille et al., 2016; Chiari et al., 2017; De Roma et al., 2017, 2018; Giorgi & Mengozzi, 2011). Nevertheless, few data are available about north-western Italy (Zoppi et al., 2012). We reported poisoning events recorded in Liguria (North West Italy) from 2009 to 2018 to improve the data. We mentioned all the samples suspected of poisoning and sent for a diagnostic investigation to the Veterinary Medical Research Institute for

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Piedmont, Liguria and the Aosta Valley (IZS-PLVA), which is the competent public veterinary institution in this region.

## 2. Materials and methods

### 2.1. Data source and management

In this study we processed data derived from the samples management system of IZS PLVA (SIGLA) since 2009: for each confirmed case, we reported to the municipality, the toxic substances detected, and the species involved. Besides a comparative analysis of the gross lesions found during necropsy in different species was performed. Finally, seasonal patterns of data submission and positive cases were analysed graphically, and a typical seasonal index was displayed.

### 2.2. Statistical analysis

All the data used in the study were reviewed and managed using Microsoft Excel 2003. The trend of prevalence across years was modelled by means of a poisson model. Temporal patterns of submitted data and positive cases found were explored graphically and typical seasonal index was displayed. Typical seasonal index is a measure of how a particular season is compared with the average season and it is calculated as the mean of the differences between the monthly observations and their moving average on a three-months period. The presence of a trend in the prevalence was assessed by means of a poisson model. The statistical analysis was carried out by means of the statistical software STATA 16.1 (Stata corp, College Station, Texas USA).

### 2.3. Study area

Liguria is in northwestern Italy, its area covers 5416.21 km<sup>2</sup> and it counts a population of 1.5 million (census, ISTAT 2017); it borders France to the east, The Mediterranean Sea to the south, Piedmont and Emilia-Romagna to the north and Toscana to the southeast. The territory is almost totally mountainous (64.9%) or hilly (34.1%) with forests covering over 60% of its area and with 61.2% of people living on the coast and concentrated in the 10 municipalities in which more than 20,000 inhabitants live. In Liguria there is an estimated population of about 228,000 dogs. Animal husbandry is a marginal activity and in the whole region there are only about 9000 cattle, 8000 sheep, 6000 goats, 2300 pigs and 5700 horses reported. Wild animals, especially wild boars and roedeers are present but foxes and wolves are relevant in forestal areas as well.

### 2.4. Toxicological analysis

A modified QuEChERS (quick, easy, cheap, effective, rugged and safe) approach was applied to speed up sample preparation and to obtain a sample extract suitable for the analysis. Toxicological screening was performed on target organs (kidney and liver) and gastric contents when the veterinarians could not exclude poisoning based on physical and gross findings of dead animals. Baits were examined and a toxicological screening performed only on those with unclear features, packaging or an unusual color, smell, content or coming from a geographic area previously subject to poisoning cases. When different visible and suspicious constituent parts were observed in baits (e.g., blue, red or violet grains), the components were analysed separately. QuEChERS approach allowed speeding up the processing of the samples, in fact, that technique needed only one-step extraction followed by a clean-up step with solid-phase dispersion extraction (dSPE), as reported in a previous work by Di Blasio et al. (2020).

Semiquantitative analytical *in-house* methods were validated for the determination of 50 toxicants in suspected poisoned baits or in tissue samples derived from a postmortem veterinarian examination (Table 1).

Due to the differences in the physicochemical properties of the

**Table 1.**  
Accredited *in-house* methods and LOQs.

| Classes                               | Compounds           | Methods       | LOQs (mg/kg) |
|---------------------------------------|---------------------|---------------|--------------|
| <i>Anticoagulant rodenticides</i>     | Acenocoumarol       | LC- MS/<br>MS | 0,05         |
| <i>Anticoagulant rodenticides</i>     | Brodifacoum         | LC- MS/<br>MS | 0,05         |
| <i>Anticoagulant rodenticides</i>     | Bromadiolone        | LC- MS/<br>MS | 0,05         |
| <i>Anticoagulant rodenticides</i>     | Chlorophacinone     | LC- MS/<br>MS | 0,05         |
| <i>Anticoagulant rodenticides</i>     | Coumachlor          | LC- MS/<br>MS | 0,05         |
| <i>Anticoagulant rodenticides</i>     | Coumafuryl          | LC- MS/<br>MS | 0,05         |
| <i>Anticoagulant rodenticides</i>     | Coumatetralyl       | LC- MS/<br>MS | 0,05         |
| <i>Anticoagulant rodenticides</i>     | Dicumarol           | LC- MS/<br>MS | 0,05         |
| <i>Anticoagulant rodenticides</i>     | Diphacinone         | LC- MS/<br>MS | 0,05         |
| <i>Anticoagulant rodenticides</i>     | Difenacoum          | LC- MS/<br>MS | 0,05         |
| <i>Anticoagulant rodenticides</i>     | Flocoumafen         | LC- MS/<br>MS | 0,05         |
| <i>Anticoagulant rodenticides</i>     | Pindone             | LC- MS/<br>MS | 0,05         |
| <i>Anticoagulant rodenticides</i>     | Warfarin            | LC- MS/<br>MS | 0,05         |
| <i>Carbamates</i>                     | Carbofuran          | LC- MS/<br>MS | 0,05         |
| <i>Carbamates</i>                     | Desmedipham         | LC- MS/<br>MS | 0,05         |
| <i>Carbamates</i>                     | Methiocarb          | LC- MS/<br>MS | 0,05         |
| <i>Carbamates</i>                     | Methomyl            | LC- MS/<br>MS | 0,05         |
| <i>Carbamates</i>                     | Oxamyl              | LC- MS/<br>MS | 0,05         |
| <i>Carbamates</i>                     | Propoxur            | LC- MS/<br>MS | 0,05         |
| <i>Molluscicides</i>                  | Methaldehyde        | GC-MS         | 5,0          |
| <i>Non-anticoagulant rodenticides</i> | α-chloralose        | LC- MS/<br>MS | 0,05         |
| <i>Non-anticoagulant rodenticides</i> | Strychnine          | LC- MS/<br>MS | 0,05         |
| <i>Organochlorines</i>                | Pindone             | GC-MS         | 5,0          |
| <i>Organochlorines</i>                | Endosulfan sulfate  | GC-MS         | 5,0          |
| <i>Organochlorines</i>                | α-endosulfan        | GC-MS         | 5,0          |
| <i>Organochlorines</i>                | β-endosulfan        | GC-MS         | 5,0          |
| <i>Organochlorines</i>                | Tetradifon          | GC-MS         | 5,0          |
| <i>Organophosphates</i>               | Chlorpyrifos ethyl  | GC-MS         | 5,0          |
| <i>Organophosphates</i>               | Diazinon            | GC-MS         | 5,0          |
| <i>Organophosphates</i>               | Ethion              | GC-MS         | 5,0          |
| <i>Organophosphates</i>               | Isofenphos          | GC-MS         | 5,0          |
| <i>Organophosphates</i>               | Malathion           | GC-MS         | 5,0          |
| <i>Organophosphates</i>               | Parathion ethyl     | GC-MS         | 5,0          |
| <i>Organophosphates</i>               | Pirimiphos methyl   | GC-MS         | 5,0          |
| <i>Organophosphates</i>               | Phorate             | GC-MS         | 5,0          |
| <i>Organophosphates</i>               | Sulfotep            | GC-MS         | 5,0          |
| <i>Organophosphates</i>               | Terbufos            | GC-MS         | 5,0          |
| <i>Organophosphates</i>               | Dimethoate          | LC- MS/<br>MS | 0,05         |
| <i>Organophosphates</i>               | Chlorpyrifos methyl | LC- MS/<br>MS | 0,05         |
| <i>Organophosphates</i>               | Methamidophos       | LC- MS/<br>MS | 0,05         |
| <i>Organophosphates</i>               | Parathion methyl    | LC- MS/<br>MS | 0,05         |
| <i>Organophosphates</i>               | Coumaphos           | LC- MS/<br>MS | 0,05         |
| <i>Organophosphates</i>               | Mevinphos           | LC- MS/<br>MS | 0,05         |
| <i>Other substances</i>               | Ethylene glycol     | GC-MS         | 5,0          |
| <i>Other substances</i>               | Pyperonil butoxide  | GC-MS         | 5,0          |
| <i>Other substances</i>               | Embutramide         | GC-MS         | 5,0          |

(continued on next page)

**Table 1.** (continued)

| Classes          | Compounds    | Methods       | LOQs (mg/kg) |
|------------------|--------------|---------------|--------------|
| Other substances | Lenacil      | LC- MS/<br>MS | 0,05         |
| Pyrethroids      | Cypermethrin | GC-MS         | 5,0          |
| Pyrethroids      | Deltamethrin | GC-MS         | 5,0          |
| Pyrethroids      | Permethrin   | GC-M-         | 5,0          |

poisons, liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) were performed to detect the substances: carbamates, organophosphates, organochlorines, pyrethroids and rodenticides anticoagulants, strychnine, alpha-chloralose, and methaldehyde.

One aliquot of purified extract was injected in a liquid-chromatograph Agilent 1100 (Agilent Technologies, St. Clara, CA, USA) coupled with tandem mass spectrometer system API4000 (Sciex, Framingham, MA, USA), operating in ESI positive and negative MRM mode and at least 2 transitions for each analyte were acquired. This instrumental technique allowed to detect anticoagulants, alpha-chloralose, carbamates, strychnine, chlorpyrifos methyl, coumaphos, methamidophos, mevinphos, parathion methyl. Another aliquot of extract was injected in a gas-chromatograph Agilent 6090 N coupled with a single quadrupole mass spectrometer Agilent 5975 system (Agilent Technologies, St. Clara, CA, USA), operating in full scan mode with EI ionization, to better detect the more volatile compounds: alpha-endosulfan, beta-endosulfan, endosulfan-sulfate, lindane, metaldehyde, piperonyl butoxide, ethylene glycol, cypermethrin, deltamethrin, permethrin I and II (Di Blasio et al., 2020).

### 3. Results

#### 3.1. General description of the recorded cases

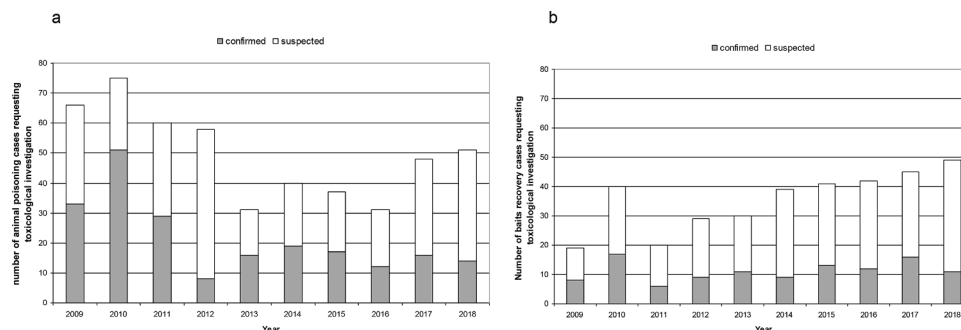
A total number of 851 samples were analysed from 2009 to 2018: 497 suspected animal poisoning and 354 suspected baits. The confirmed animal poisoning cases were 43.2% while at least one toxic substance was found 31.1% of baits.

Both the carcasses (all their parts, including gastric lavage or vomit) and baits samples were treated as suspected. The baits were delivered to the laboratories accompanied by specific modules according to the above mentioned specific Italian law against deliberate poisoning in animals. The distribution of confirmed and suspected samples per year is reported in Fig. 1a and b.

Although the graphs suggest a trend, the Poisson model shows that no statistically significant trend is present.

#### 3.2. Seasonal and geographical distribution

The graphs in Fig. 2(a) and (b) show the typical seasonal index



**Fig. 1.** Distribution per year of suspected animal poisoning (a) and baits recovery cases (b) requesting toxicological investigations between 2009 and 2018 and the relative number of those where toxic agents were confirmed.

calculated for suspected and confirmed poisoning events. A seasonal pattern is not noticeable although the majority of suspected samples were submitted in January, March, April, and August and the confirmed samples were found mostly in January, March, and August.

The geographical distribution of positive animal samples and poisoned baits in ligurian region is presented in Fig. 3a and b: a higher density per municipality of confirmed cases is represented with a darker color. The coastal municipalities, the provincial capitals and, in general, the most densely populated areas are more involved in poisoning animal cases and poisoned baits recovery occurrences.

#### 3.3. Toxic compounds involved

Out of 308 of animal poisoning cases 79.2% was anticoagulant rodenticides, 7.1% non-anticoagulant rodenticides, 5.2% molluscicides, 3.6% organophosphates, 2.6% organochlorines (2.6%), 1% carbamate-sand, 1% pyrethroids (Table 2). In 155 of the analysed sample a single toxic was used whereas in 60 samples a mixture of several compounds was detected: each compound detected in mixtures was counted in its own category.

As reported in Table 2, coumatetralyl (41,4%), brodifacoum (22,5%), bromadiolone (17,6%) and difenacoum (13,5%) were the most involved compounds in anticoagulant rodenticides class;  $\alpha$ -chloralose (90.9%) in non-anticoagulant rodenticides; methaldehyde was the only detected compound in molluscicides.

Out of 171 poisoned baits samples, 48.5% of toxic compounds was anticoagulant rodenticides, 13.4% organophosphates 12.3% organochlorines, 7.0% non-anticoagulant rodenticides, 5.9% molluscicides, 5.9% carbamates, 3,5% the synergist piperonyl butoxide and 2.3% pyrethroids.

#### 3.4. Involved species

The percentage distribution of species for suspected poisoning cases were found with the following decreasing order: dogs (34.2%), cats (33.6%), synanthropic birds like mallards (*Anas platyrhynchos*) and pigeons (*Columba livia*) (17.7%), livestock (bees, donkeys, cattle, goats, rabbits, horses, sheep, chickens and pigs) (8.2%) and wild animals (6.3%).

Toxicological screening confirmed poisoning for: 41% of dogs, 39% of cats and 15% of synanthropic birds, 4% of wild animal and 1% of livestock.

Poisoning in dogs and cats was mainly caused by anticoagulant rodenticides (68.5% and 82.5% respectively): the 62.9% of positive dogs and 45.5% of positive cats showed abdominal, thoracic or subcutaneous hemorrhages, while in the remaining cases lesions were not detected or pathological findings were related to pre-existing diseases. Other toxicant detected were molluscicides (14.1%), insecticides (13.1%), non-anticoagulant rodenticides (13%), but no significant lesions were found in dogs and cats poisoned with those toxicants.

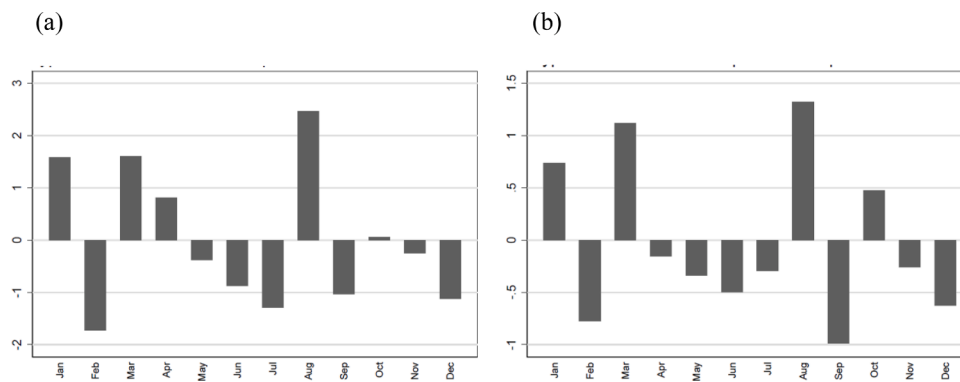


Fig. 2. Typical seasonal index of samples (a) and positive samples (b) submitted in 2009–2018.



Fig. 3. Number of confirmed animal poisoning cases (a) and number of confirmed poisoned baits recovery cases (b).

From 2009 to 2018, 87 episodes of suspected poisoned volatiles were found but only 32 were confirmed. In particular, 41 cases of suspected poisoning of volatiles like mallard, synanthropic birds and sparrows were examined. In 32% of these samples at least one toxicant was found:  $\alpha$ -chloralose (7%) and brodifacoum (24%). In 5 cases botulinum toxin was detected and no hemorrhagic lesions were found.

As for pigeons only 19 cases out of 46 were confirmed. Anticoagulant rodenticides were the most present toxicants also in pigeons (47.8%) but hemorrhagic lesions were found only in one case due to bromadiolone and difenacoum. In all the other episodes non-anticoagulant rodenticides was detected: 30.4% was  $\alpha$ -chloralose.

Only a few cases involving wild animals were found (5 foxes, 4 wolves and 3 wild boars) and their necropsy exam showed hemorrhagic lesions, due to confirmed intake of toxic substances (i.e. bromadiolone and brodifacoum). Only 3 cases out of the 41 samples of farm animals collected showed the presence of anticoagulant rodenticides.

#### 4. Discussion

This study shows that, despite the legal prohibition, deliberate animal poisoning is still present and steady in Liguria. As other authors previously pointed out (Bille et al., 2016), the requests of toxicological screening have grown. Since the entry into force of the ordinance in 2009, which has set the obligation to perform necropsy and toxicological investigations for free, in Liguria an average of 80 suspected poisoning cases per year were submitted to our laboratories.

As conversely occurred in other Italian regions, the use of banned substances was limited in frequency and not linked to specific local context (table 2).

In particular, the illegal use of endosulfan was already reported in Italy (Chiari et al., 2017; De Roma et al., 2017; Di Blasio et al., 2020), probably linked to some residual rural stock after the ban of the sale and the use of this substance respectively in 2012 and 2013, (Di Blasio et al., 2020).

As for the geographical aspect, animal poisoning and baits scattering appears to be present similarly along the coast where the most populated municipalities are located. Actually, this aspect could confirm that the

most important causes of the intentional use of poisoned baits are neighborhood problems or cohabitation with synanthropic animals (Sánchez-Barbudo, Camarero & Mateo, 2012). Baits scattering could be a severe risk for other animal species or humans, especially children, and could create a real problem of environmental pollution. It is important to search pyrethroids because it might be dangerous for humans, even if it is practically nontoxic to birds and mammals. Children may be more sensitive to pesticides than adults. For example, they may spend more time near the floor. They may also be more likely to touch their mouths after being in contact with treated surfaces or pets (Cross, Bond, Buhl & Jenkins, 2017). In our competent territory pyrethroids (i.e., permethrin and cypermethrin) were found in baits packaged with only pyrethroids and/or its synergist (piperonyl butoxide); in some cases, the synergist was detected alone in baits or mixed with inert harmful materials (i.e. blades) (Di Blasio et al., 2020).

Although poisoning is more frequent in January, March, April, and August, the distribution of suspected and confirmed poisoning cases doesn't show a typical seasonal index. This data are different from the information about central Italian regions (Amorena et al., 2009) and north-eastern Italian regions (Bille et al., 2016), where a positive trend of poisoning was noticed from March to June and in September. Liguria is a touristic region and its population can double during Christmas, Easter and summer holidays, as it often occurs along all the Italian coasts.

Pets were the most involved species (roughly 40%) among the poisoned animals reported. This trend was previously analysed for Europe and Italy (Berny et al., 2010; Bille et al., 2016) and supported by the Poison Control center of Milan (Caloni, Cortinovis, Rivolta & Davanzo, 2012) which reported an increase in the poisoning of pets, especially cats, due to a growing number of feline colonies and owned cats. The "second-generation" anticoagulants like brodifacoum and bromadiolone are highly toxic to nontarget species (e.g. dogs, cats or wildlife) also after a single feeding. So, pets are very sensitive to anticoagulants, and hemorrhages were frequently detected. However, it is very important to point out that the number of poisonings is higher in cats than in dogs. Synanthropic birds were the most involved species after pets: pigeons are often poisoned because of hygiene problems due to their presence in urban areas.

**Table 2.**  
Toxic agent detected in animal poisoning and poisoned baits recovery cases between 2009 and 2018.

| toxic agent                         | Animals poisoning    |                                    | Poisoned baits       |                                    |
|-------------------------------------|----------------------|------------------------------------|----------------------|------------------------------------|
|                                     | Number of detections | % on subtotal for chemical classes | Number of detections | % on subtotal for chemical classes |
| <i>Arbamates</i>                    |                      |                                    |                      |                                    |
| Desmedipham                         | 0                    | 0,0                                | 1                    | 10,0                               |
| Methiocarb                          | 0                    | 0,0                                | 1                    | 10,0                               |
| Methomyl                            |                      |                                    |                      |                                    |
| <b>Propoxur*</b>                    | 3                    | 100,0                              | 7                    | 70,0                               |
|                                     | 0                    | 0,0                                | 1                    | 10,0                               |
| <i>Total</i>                        | 3                    | 1                                  | 10                   | 5,9                                |
| <i>Molluscicides</i>                |                      |                                    |                      |                                    |
| Methaldehyde                        | 16                   | 100,0                              | 10                   | 100,0                              |
| <i>Total</i>                        | 16                   | 5,2                                | 10                   | 5,9                                |
| <i>Organochlorines</i>              |                      |                                    |                      |                                    |
| <b>Endosulfan*</b>                  | 8                    | 100,0                              | 21                   | 100                                |
| <i>Total</i>                        | 8                    | 2,6                                | 21                   | 12,3                               |
| <i>Anticoagulant rodenticides</i>   |                      |                                    |                      |                                    |
| Brodifacoum                         | 55                   | 22,5                               | 16                   | 19,3                               |
| Bromadiolone                        | 43                   | 17,6                               | 26                   | 31,3                               |
| Chlorophacinone                     | 2                    | 0,8                                | 5                    | 6,0                                |
| Coumatetralyl                       | 101                  | 41,4                               | 14                   | 16,9                               |
| Dicumarol                           | 0                    | 0,0                                | 2                    | 2,4                                |
| Difenacoum                          | 33                   | 13,5                               | 16                   | 19,3                               |
| Flocoumafen                         | 5                    | 2,1                                | 0                    | 0,0                                |
| Warfarin                            | 5                    | 2,1                                | 4                    | 4,8                                |
| <i>Total</i>                        | 244                  | 79,2                               | 83                   | 48,5                               |
| <i>Organophosphates</i>             |                      |                                    |                      |                                    |
| Diazinon                            | 0                    | 0,0                                | 4                    | 17,4                               |
| Dimethoate                          | 0                    | 0,0                                | 1                    | 4,3                                |
| Malathion                           | 3                    | 27,3                               | 6                    | 26,1                               |
| Methamidophos                       | 0                    | 0,0                                | 3                    | 13,0                               |
| <b>Parathion*</b>                   | 2                    | 18,2                               | 2                    | 8,7                                |
| Phorate                             | 1                    | 9,1                                | 0                    | 0,0                                |
| Pirimiphos methyl                   | 4                    | 36,3                               | 5                    | 21,8                               |
| <b>Sulfotep*</b>                    | 1                    | 9,1                                | 2                    | 8,7                                |
| <i>Total</i>                        | 11                   | 3,6                                | 23                   | 13,4                               |
| <i>Other rodenticides</i>           |                      |                                    |                      |                                    |
| $\alpha$ Chloralose                 | 20                   | 90,9                               | 11                   | 91,6                               |
| <b>Strychnine*</b>                  | 2                    | 9,1                                | 1                    | 8,4                                |
| <i>Total</i>                        | 22                   | 7,1                                | 12                   | 7,0                                |
| <i>Pyrethroids</i>                  |                      |                                    |                      |                                    |
| Deltamethrin                        | 0                    | 0,0                                | 1                    | 25,0                               |
| Permethrin                          | 3                    | 100                                | 3                    | 75,0                               |
| <i>Total</i>                        | 3                    | 1                                  | 4                    | 2,3                                |
| <i>Other substances</i>             |                      |                                    |                      |                                    |
| Ethylene glycol                     | 0                    | 0,0                                | 2                    | 25,0                               |
| Pyperonil butoxide                  | 1                    | 100                                | 6                    | 75,0                               |
| <i>Total</i>                        | 1                    | 0,3                                | 8                    | 4,7                                |
| Total analyte in the positive cases | 308                  | 100,0                              | 171                  | 100,0                              |

\* Banned substances in Europe according to current legislation.

Unlike other species, analysis of pigeons is usually required when sudden death of several birds take place, and the laboratories are called to check acute poisoning and causes of infections. As observed in other regions (Di Blasio et al., 2020),  $\alpha$ -chloralose is surely the most common toxicant for killing pigeons (Segev, Yas-Natan, Shlosberg & Aroch, 2006), probably because it is not subjected to commercial limitations and is easy to find.  $\alpha$ -chloralose as toxic compound is a moderately toxic compound to mammals, with a high potential on bioaccumulation, but it is toxic and fatal for birds, birds eaters or predators, such as domestic dogs and cats (Di Blasio et al., 2020).

Detection of brodifacoum in birds and other wild animals could be due to a fraudulent poisoning or to environmental pollution. In fact, this substance is scarcely degradable and has a long half-life (DT50 at 20 °C: 157 days; DT 50 at 12 °C: 298 days); it was considered almost persistent in the soil where it can remain for a long time (DT50 over 1 year) (Reg.

CE 2012/528).

Cases involving wild animals represented only 4% of the total amount of the present study, but it might have been underestimated because the recovery of dead animals in wild areas is less frequent than in a rural or urban contest. This critical issue highlighted the necessity to strengthen the rescue activities and extend the toxicological screening to all dead wild animal recovered, even if no baits were detected to suspect a poisoning case. Moreover, death of wild animals might occur far from the place of exposure when poisoning was caused by slow-acting compounds such as some anticoagulant rodenticides.

Finally, poisoning events of livestock are negligible and the only three confirmed cases were probably due to an accidental intake of anticoagulants because of rodent control in the stables. Anticoagulant rodenticides, especially the second and third generation, were said to be involved in 79.2% of animal poisoning cases and in 48.5% of examined baits. Actually, we can reasonably assume that these toxicants are the most important causes of animal poisoning: they induce hemorrhagic syndromes by inhibiting the recycling of vitamin K1 and tend to accumulate in the liver and death usually occurs a few days after ingestion during bleeding events (Caloni, Cortinovis, Rivolta & Davanzo, 2016; Muscarella, Armentano, Iammarino, Palermo & Amorena, 2016; Sánchez-Barbudo et al., 2012). The most widespread authorised rodenticides are the anticoagulants (Cabella, Bellomo & Rubbiani, 2015), and in many cases a misuse without following good practices can cause accidental intake and poisoning of a non-target species.

## 5. Conclusions

This study highlights that intentional animal poisoning is a widespread problem in Liguria. In fact, although institutions have shown some interests toward the problem by issuing a prohibitive ordinance, poisoning of domestic and wild animals is still present, and it is growing in the north west area of Italy. This phenomenon is not only harmful towards animal health: poison can spread in the environment, polluting the soil and surface waters, or coming into direct contact with people, especially children, exposing them to a serious risk for their health. This demonstrates that there is a need to strengthen the monitoring and control activities by the health authorities, and to improve information. In addition, it is necessary to develop a diagnostic protocol and a common toxicology panel test on a national scale.

The effectiveness of the ministerial ordinance can be increased rising the awareness of the actors involved and improving their technical knowledge. For this reason the Italian Ministry of Health created a website called 'National Poisonings Portal' in cooperation with IIZZSS: the system allows to have an online management of the poisoning cases, from the report of the suspected cases to the definitive diagnosis, as well as the electronic submission of the documents required by the legislation in force to the competent authorities.

## Ethical Statement for Solid State Ionics

Hereby, I Rosa Avolio consciously assure that for the manuscript Retrospective data analysis of animal poisoning events in Liguria the following is fulfilled:

- 1) This material is the authors' own original work, which has not been previously published elsewhere.
- 2) The paper is not currently being considered for publication elsewhere.
- 3) The paper reflects the authors' own research and analysis in a truthful and complete manner.
- 4) The paper properly credits the meaningful contributions of co-authors and co-researchers.
- 5) The results are appropriately placed in the context of prior and existing research.

- 6) All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.
- 7) All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.

The violation of the Ethical Statement rules may result in severe consequences.

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I agree with the above statements and declare that this submission follows the policies of Solid State Ionics as outlined in the Guide for Authors and in the Ethical Statement.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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