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POTENTIAL HEALTH RISK TO HUMANS RELATED TO ACCUMULATION OF BRODIFACOUM AND BROMADIOLONE IN THE WHEAT GROWN ON RODENTICIDE CONTAMINATED SOIL

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ABSTRACT

The aim of this study was to determine in a model experiment the potential residues of bromadiolone and brodifacoum in the wheat grown on soil treated with these rodenticides and to compare them with the respective acceptable daily intake (ADI) in order to obtain information lacking in the scientific literature. The study focused on the level of residues of chronic rodenticides Broder G, with the active ingredient brodifacoum, and DERATION G, with the active ingredient bromadiolone, in wheat *(Triticum* spp.). The preparations were used in the form of granular bait. In the wheat grown on the soil treated with 100 g.m^{-2} of the preparation BRODER G, the brodifacoum residues ranged from 0.012 to 0.0218 mg.kg^{-1}, while the treatment of soil with 500 g.m^{-2} resulted in residues ranging between 0.0344 and 0.0436 mg.kg^{-1}. When using the preparation DERATION G, bromadiolone residues ranged between 0.012 and 0.018 mg.kg^{-1} after the treatment of soil with 100 g.m^{-2} and between 0.030 and 0.0428 mg.kg^{-1} after the treatment with 500 g.m^{-2}. We observed that the acceptable daily intake was exceeded significantly in all of the cases and the residual levels depended on the rodenticide dose. In the case of brodifacoum, the ADI was exceeded more than 700-fold at a dose of 100 g.m^{-2} and more than 1400-fold at a dose of 500 g.m^{-2} of soil. With bromadiolone, the ADI was exceeded 150-fold at a dose of100 g.m^{-2} and more than 350-fold at a dose of 500 g.m^{-2}. This indicates the risk to consumers from such crops.

Key words: brodifacoum; bromadiolon; cereal crop; food safety; residues

INTRODUCTION

Targeted, unauthorised or unintentional application of chemicals and their spreading and persistence in the environment presents risks to humans and other animals, and has negative effects on biodiversity in general.

Managing populations of commensal rodents is essential. By sharing our environment, they can transmit to humans, directly or indirectly, more than 40 zoonotic patho-

gens, such as Yersinia pestis, hantavirus and Leptospirosis species. They can cause the destruction or degradation of cereals, accounting for nearly 10 % of the grain crops in the world, with considerable variation depending upon the country [2].

Due to the neophobic behaviour of rodents, the only really effective molecules that can be used to manage their populations are anticoagulant rodenticides (ARs), sometimes also referred to as chronic rodenticides. Their delayed action, with mortality occurring several days after consumption, makes them effective in controlling neophobic species. ARs are usually classified into two generations. The activity of ARs of the first generation (warfarin) is now largely limited by the genetic resistance phenomenon. The second generation of ARs (bromadiolone, difenacoum, brodifacoum, flocoumafen and difethialone), sometimes referred to as superwarfarins, are chemically modified forms of warfarin where substituted phenyl rings are attached to the 4-hydroxycoumarin moiety [19]. Second generation ARs have been used since the 1980s for pest management. They are currently the most widely used rodenticide compounds and are authorized through various legislative provisions.

Some recent research makes an effort to implement a third generation of ARs which is based on the stereochemistry concept (difenacoum enriched in trans-isomers), which would be efficient against resistant strains of rodents and be less persistent and thus less involved in secondary poisonings [9, 10].

These anticoagulant compounds used as rodenticides inhibit vitamin K 2,3-epoxide reductase, affecting vitamin K-dependent coagulation factors in the liver and causing spontaneous internal bleeding and, at lethal doses, death [39].

In the past, the availability of an effective antidote, vitamin K1, was considered an advantage of anticoagulant rodenticides. Early administration of this antidote, i. e. before haemorrhage affects the vital centres, will prevent death. However, while the anticoagulant actions of superwarfarins are ultimately responsible for their lethality, additional targets exist that could lead to pathological consequences or contribute to their toxicity. In addition to proteins in the coagulation cascade, vitamin K is a cofactor for several other proteins throughout the body, and modeling studies suggest there may be over 100 targets for GGC (γ-glutamyl carboxylase). Lower vitamin K levels will therefore reduce the GGC-dependent carboxylation of these proteins, including osteocalcin, a hormone involved in energy metabolism, fertility, brain development, and bone remodeling [27] and the matrix Gla protein, important for vascular decalcification [34]. In addition to their roles in clotting, vitamin K-dependent proteins are vital to several other processes, including ones that help maintain the normal brain function and homeostasis [20]. Thus, unlike warfarin overdose for which 1—2 days of treatment with vitamin K is effective, treatment of superwarfarin poisoning with vitamin K is limited by its extremely high cost and can require daily treatment for a year or longer.

Due to their increased tissue persistency and bioaccumulation in the environment, non-target poisoning by second generation ARs is commonly described in wildlife. Different species are affected by this exposure, such as red foxes, European minks, bobcats and various raptors such as red kite, buzzards, kestrels, peregrine falcons, and eagle owls [26, 30]. Besides potential adverse sublethal effects on wildlife, there is also a concern for human exposure through consumption of meat from wild game animals that carry brodifacoum residues [4].

With regard to the high toxicity of chronic rodenticides to animals, monitoring of their influence on other non-target species has been initiated. B o o t h et al. [3] described acute toxicity of brodifacoum to invertebrates, such as snails and terrestrial species of crabs. E a s o n et al. [13] examined poisoned wild pigs and goats for the presence of brodifacoum residues and B o o t h et al. [3] investigated toxic effects of brodifacoum on earthworms.

Because of the well-known use-related risks, ARs were identified by the European Union as candidates for future comparative risk assessment and substitution.

Regulation (EU) No. 528/2012 [32] which concerns the making available on the market and use of biocidal products state conditions for approval and authorization of biocidal products, including products used for the control of mice, rats or other rodents, by means other than repulsion or attraction. Since brodifacoum and bromadiolone fulfil the criteria set in Article 5(1) of this Regulation, the approval of these rodenticides in product type 14 should normally not be renewed. However, this raised an issue of sufficiently effective alternatives that would present no practical or economical disadvantages. On the basis of an input from stakeholder organisations, companies, non-governmental bodies, independent experts and na-

tional bodies, the Biocidal Products Committee (BPC) presented in 2016 an opinion on the application for renewal of the approval of the active substance: Brodifacoum, Product type: 14, ECHA/BPC/113/2016, and Bromadiolone, Product Type 14, ECHA/BPC/111/2016, both adopted 16th June 2016 [14, 15]. This opinion was based on the conclusion that currently no significant and effective alternative to anticoagulant rodenticides is readily available. Non-chemical controls or prevention methods for rodent control, such as mechanical, electrical or glue traps, may not be sufficiently efficient and may raise further questions as to whether they are humane or whether they cause unnecessary suffering to rodents. Alternative active substances approved for use as rodenticides may not be suitable for all user categories or efficient for all rodent species. Rodent control currently relies largely on the use of anticoagulant rodenticides, the non-approval of which could lead to insufficient rodent control. This may not only cause significant negative impacts on human or animal health or the environment, but also affect the public's perception of its safety with regard to exposure to rodents or the security of a number of economic activities that could be vulnerable to rodents, resulting in economic and social consequences. On the other hand, the risks to human health, animal health or the environment arising from the use of products containing brodifacoum and bromadiolone can be mitigated if they are used according to certain specifications and conditions.

Specific conditions for the use of rodenticide preparations based on brodifacoum and bromadiolone were set by the Commission Implementing Regulations (EU) 2017/1380 and 2017/1381 [7, 8].

Other rodenticides that currently are registered to control rodents are bromethalin, cholecalciferol and zinc phosphide. These compounds are not anticoagulants. Each is toxic in other ways [17].

Brodifacoum is a bromylate derivative of hydroxycoumarin that was used for the first time in 1975 to control rodents resistant to coumarin [25]. The solubility of brodifacoum in water is 0.004 $mg.l^{-1}$ [23]. Brodifacoum has been supported and evaluated as a rodenticide in the following use situations: in and around building and in sewers. The target species are brown rat, black rat and the house mouse. This rodenticide is not readily biodegradable. It is hydrolytically stable to hydrolysis (Th > 1 year). The degradation time DT50 value of 157 days indicate that brodifacoum would be persistent and immobile in soil. The exposure to the groundwater is unlikely. Under basic conditions (high pH), brodifacoum is not likely to be adsorbed onto soils or sewage sludge due to the ionisation of the molecule; whereas under acidic conditions (low pH), brodifacoum is likely to be adsorbed onto soils or sewage sludge as the molecule is in its neutral or non-ionised form [11].

Bromadiolone was used for the first time in 1976 also to control rodents resistant to warfarin. It is more effective and at the same time less toxic than other chronic anticoagulant rodenticides of the 1st and 2nd generation [28]. Bromadiolone has been evaluated as a rodenticide against rats and mice for the following use patterns: in and around buildings (professional and non-professional use), sewers (professional use only), open areas (professional use only) and waste dump (landfill) perimeters (professional use only). The solubility of bromadiolone in water is 0.002 $mg.l^{-1}$ [23]. Bromadiolone is not readily biodegradable under environmentally relevant conditions or during sewage treatment processes. It is also not inherently biodegradable. No hydrolysis was found at pH 7, or 9, so hydrolysis of bromadiolone is not expected to be a significant process in the environment. Bromadiolone is quickly degraded in soil under aerobic conditions with an estimated DT50 value between 4 and 53 days (at 12 °C, extrapolated from 20 and 25 °C, LiphaTech), however degradation led to the formation of unidentified soil metabolites which persisted in significant quantities for > 1570 days. Laboratory soil column leaching and aged leaching studies performed by LiphaTech indicated that bromadiolone and any potential degradation products, even if released indirectly to soil in small quantities, are not likely to move through the soil profile and are unlikely to reach groundwater in significant quantities [12].

Commission Directives 2009/92/EC and 2010/10/EU [5, 6] dealt with the relevant issues by drawing attention to high persistence, bioaccumulation and toxicity of ARs. The estimated daily exposure that will not cause harmful effects throughout the human life is referred to as Acceptable Daily Intake (ADI) in $mg.kg^{-1}$ body weight. ADI for brodifacoum has been estimated as 0.0000005 $mg.kg^{-1}.day^{-1}$ which corresponds to NOEL (No Observable Effect Level) equal to 0.001 $mg.kg^{-1}.day^{-1}$. ADI for bromadiolone has been estimated to 0.000002 $mg.kg^{-1}.day^{-1}$, which corresponds to NOEL for bromadiolone equal to 0.004 $mg.kg^{-1}.day^{-1}$ [35].

EFSA [16] has reviewed the maximum residue levels (MRLs) currently established at the European level accord-

ing to Article 12 of Regulation (EC) No. 396/2005 [31] for the pesticide active substance bromadiolone. Considering that this active substance was not authorised for use on edible crops within the EU, no MRLs were established by the Codex Alimentarius Commission (codex maximum residue limits) and that no import tolerances were notified to EFSA, residues of bromadiolone were not expected to occur in any plant or animal commodity. Even though information on the limit of quantification (LOQ) of bromadiolone was provided by the European Union Reference Laboratories for Pesticide Residues, the available data were not sufficient to derive a residue definition for enforcement against potential illegal uses.

Most studies related to the risk of ARs focused on the analysis of rodenticides in biota. Probably, because of their low solubility in water, very few studies investigated the presence of rodenticides in water. Recently some studies related to the authorised use of ARs indicated that anticoagulants can reach sewage waters that are treated in wastewater treatment plants (WWTP) and pass to treated water and sewage sludge [22, 23, 33]. Thus they may be discharged into rivers or applied to soil manured with sludge.

With regard to the risk of the presence of ARs in agricultural soil and their translocation to crops, even with the professional use of anticoagulant rodenticides, one must also consider the potential changes in the integrity of pellets after being subjected to precipitations. Also the rules of correct agricultural practice may occasionally be violated. Thus grain crops, for example wheat, which are used directly for human consumption (breadstuff's), or as feed for farm animals (concentrate), may be exposed to ARs. For the 2019 crops, FAO's forecast for world wheat production is 757 million tonnes, which would place this year's output 4 percent above the 2018 level [18].

The aim of our study was to investigate the potential risk to humans related to the consumption of wheat grown on soil treated with second generation ARs (brodifacoum, bromadiolone).

MATERIALS AND METHODS

The rodent control preparations BRODER G and DERATION G (granular baits) used in this study are based on anticoagulant rodenticides of the 2nd generation, brodifacoum and bromadiolone, respectively. The study was carried out in five subsequent years 2013—2017. Wheat *(Triticum* spp., order *Cyperales,* family *Poaceae)* was sown to open boxes of dimensions 1x 1 x 0.5 m, that contained loam soil suitable for growing wheat.

Two open boxes were used for each of two AR doses and additional two were used as a control. The boxes were placed outside and were exposed to precipitations.

In the second half of March of each year, rodenticides were placed onto the surface of the soil in the respective boxes at doses of 100 $g.m^{-2}$ and 500 $g.m^{-2}$ of each preparation and worked into the top 5 cm layer. Then 7.5 g of wheat grains *(Triticum* spp.) were sown to each box to the depth of about 25—50 mm. No other agrotechnical measures were taken throughout the vegetation period. In early August, at harvest time, the wheat was scythed manually and respective samples (whole spikes) were collected and homogenized. Control samples were obtained from wheat grown on untreated soil. This procedure was repeated for an additional 4 years using fresh soil free of rodenticide residues.

The samples collected were thoroughly homogenized and 10 g aliquots were extracted for 15 min with 25 ml of a mixture of chloroform + acetone (1 : 1). The solvent was then decanted through a filter paper and the extraction was repeated once more with a fresh solvent. The extracts were combined and the solvent was evaporated at approximately 40 °C in a rotating vacuum evaporator. The residuum was dissolved in l ml of methanol and 1 ml of the mobile phase was added to remove co-extracted proteins. The solution was then transferred into a centrifugation tube containing n-hexane and centrifuged for 10 min at 3500 rpm. The cleared lower layer was filtered and used for HPLC chromatographic analysis (Agilent, 1200 Series HPLC System, Agilent Technologies, USA).

The chromatographic analysis was carried out on a column LiChrospherR 100 RP-18 (5 gm) with mobile phase acetonitrile (A) + acetate buffer, pH 4.6 (B), ratio 60 + 40, at a flow rate of l $ml.min^{-1}$, injected aliquot 10 gl and UV detection at 265 and 310 nm.

The statistical evaluation was carried out using GraphPad Prism Anova. The P threshold value for statistical significance was set at $P < 0.05$.

RESULTS

Residues of brodifacoum and bromadiolone determined between 2013 and 2017 are presented in Table 1. The treatment of soil with 100 g.m^{-2} dose of preparation BRODER G resulted in wheat residual levels ranging between 0.012 and 0.0218 mg.kg^{-1} while the dose of 500 g.m^{-2} produced residues ranging between 0.0344 and 0.0436 mg.kg^{-1}. Treatment of soil with 100 g.m^{-2} of DERATION G resulted in residues in wheat between 0.012 and 0.018 mg.kg^{-1} while the dose of 500 g.m^{-2} produced residual levels between 0.030 and 0.0428 mg.kg^{-1}.

A comparison with ADI for brodifacoum in humans (0.0000005 mg.kg^{-1}.day) for a 60 kg individual (0.00003 mg.kg^{-1}.day) showed that the rodenticide dose of 700 g.m^{-2} soil caused a significant increase in brodifacoum in wheat that exceeded the respective ADI 400-fold in 2014 and more than 700-fold in 2015 (Table 1).

A dose of 500 g.m^{-2} soil of BRODER G resulted in more than 1100-fold higher level in 2014 and more than 1400-fold higher level in 2015 and 2017 in comparison with ADI for a 60 kg man.

The precipitations in the area of the study varied considerably between individual years and months. In the years 2014, 2015 and 2017, they were distributed relatively evenly, in 2013 they were more intensive in the second half of the study period and in 2016 most of them occurred in April and May. No correlation was found between the residues in wheat and the mean monthly precipitations during the growing season (April—August).

A comparison with bromadiolone ADI for humans (0.000002 mg.kg^{-1}.day^{-1}) considering a 60 kg individual (0.00012 mg.kg^{-1}.day^{-1}) showed that the rodenticide dose of 100 g.m^{-2} soil resulted in 100-fold higher level in 2015 and 150-fold higher level in 2016 than the level acceptable for 60 kg individual (Table 1).

Table 1. Residues of brodifacoum and bromadiolone in control wheat and wheat grown on soil treated with these rodenticides at doses 100 g.m^{-2} and 500 g.m^{-2}

Years	2013	2014	2015	2016	2017
Mean monthly rainfall (mm) during the study period (April—August)	73.6	120.8	52.6	89	89.4
Control ADI 60 kg man, brodifacoum mg.day^{-1}			**0.00003**		
Residues of brodifacoum in wheat И BRODER G dose 100 g.m^{-2} soil					
Residues of brodifacoum mg.kg^{-1} in control wheat	0	0	0	0	0
Residues of brodifacoum mg.kg^{-1} in treated wheat	0.0196*	0.012*	0.0218*	0.02*	0.0178*
Residue—ADI multiple	653.3	400	726.6	666.6	593.3
Residues of brodifacoum in wheat—BRODER G dose 500 g.m^{-2} soil					
Residues of brodifacoum mg.kg^{-1} in control wheat	0	0	0	0	0
Residues of brodifacoum mg.kg^{-1} in treated wheat	0.040*	0.0344*	0.043*	0.038*	0.0436*
Residue—ADI multiple	1333.3	1146.6	1433.3	1266.6	1453.3
Control ADI 60 kg man, bromadiolone mg.day^{-1}			**0.00012**		
Residues of bromadiolone in wheat—DERATION G dose 100 g.m^{-2} soil					
Residues of bromadiolone mg.kg^{-1} in control wheat	0	0	0	0	0
Residues of bromadiolone mg.kg^{-1} in treated wheat	0.017*	0.014*	0.012*	0.018*	0.014*
Residue—ADI multiple	141.6	116.6	100	150	116.6
Residues of bromadiolone in wheat—DERATION G dose 500 g.m^{-2} soil					
Residues of bromadiolone mg.kg^{-1} in control wheat	0	0	0	0	0
Residues of bromadiolone mg.kg^{-1} in treated wheat	0.040*	0.03*	0.036*	0.0428*	0.035*
Residue—ADI multiple	333.3	250	300	356.6	291.6

*—significant at $P < 0.001$

The dose of 500 g.m^{-2} soil of DERATION G resulted in 250-fold higher level in 2014 and more than 350-fold higher level in 2016 in comparison with the ADI for 60 kg man.

DISCUSSION

Anticoagulant poisons have an ongoing history of worldwide use for the control of pest vertebrates, particularly rodents. Control of rodents is important not only to reduce the very high economic losses caused by rodents in both animal and plant production but also the losses associated with disease transfer which are frequently much higher. Balancing benefits of rodent control and the potential costs of applying anticoagulant rodenticides to the environment remains a challenge.

Superwarfarins like BDF and DiF represent an emerging threat to human life. The increased use of these agents has raised the risk of accidental bait ingestion or incidental exposure to residual material present in the environment following widespread dispersals [19].

There is also a significant risk of unintentional exposure to superwarfarins as a result of their use to eradicate rodents. In 2001, close to 20 tons of rodent bait containing 0.002 % BDF, for a total of 360 g, was released into the environment owing to a transport accident on the east coast of South Island in New Zealand [29]. Measurements were performed immediately after the spill and up to 21 months later. While the levels in water and sediment declined to below detectable limits, within 9 days brodifacoum was detectable in the shellfish for up to 21 months after the spill and calculated to require up to 31 months before the levels were acceptable for human consumption. In 2010, 700 kg of similar bait was accidentally dropped into Lake Kiririua in New Zealand [21]. It was estimated that 10 bags sank intact, and water and sediment measurements did not detect significant brodifacoum up to 1 month later. The absence of brodifacoum in the few animals tested may be due to its poor solubility in water. Although residual brodifacoum levels are considered safe with respect to their effects on anticoagulation, they may have actions that occur at significantly lower levels.

Generally, the currently used chronic anticoagulant rodenticides are toxic to both humans and other animals. Their slow onset of effect and death of rodents after several days results in their increased effectiveness. The concentration of the active ingredients in the bait in rodenticides of the 2nd generation, e. g. bromadiolone or brodifacoum, is 0.005 %. Such baits act sufficiently after acceptance of only one dose, as 3 g of the bait have already lethal effects. Preparations of the 3rd generation, e. g. difenacoum, contain 0.0025 % of the active ingredient, which is below the threshold of taste sensitivity of rats which equals to 0.005 % [37].

Our investigations confirmed the presence of residues of ARs dependent on the dose of bait incorporated into soil.

Incorporation of BRODER G at a dose of 100 g.m-^{2} of soil resulted in the residues of brodifacoum in wheat that ranged between 0.012 and 0.0218 mg.kg^{-1} and the dose of 500 g.m^{-2} of the soil produced residues between 0.0344 and 0.0436 mg.kg^{-1}.

After incorporation of DERATION G at a dose of 100 g.m^{-2} of soil, the residues of bromadiolone in wheat ranged between 0.012 and 0.018 mg.kg^{-1} and at a dose of 500 g.m^{-2} of soil between 0.030 and 0.043 mg.kg^{-1}.

From among all descriptions dealing with the use of anticoagulant rodenticides those that investigated their elution from soil and potential residues in plants date back mostly to the eighties and nineties of the past century [1, 24, 36, 38].

A study was carried out with ^{14}C-bromadiolone in four types of soil. With a soil rich in clay and organic compounds, bromadiolone stayed in the superficial layer and scarcely moved. However, in soil poor in clay and organic compounds, 67 % of the added bromadiolone was eluted [36]. Since anticoagulant rodenticides are not intended for direct application to growing crops, no residues in plant food stuffs are expected. Unlike conventional crop protection products, which must be applied over relatively large crop areas, rodenticides are applied to discrete sites in the form of low concentration baits. Even if the bait is spilled, it will not be taken up by plants. H a l l and P r i e s t l e y [24] monitored the metabolism of ^{14}C-brodifacoum in soil under aerobic conditions after applying it at a nominal rate of 0.4 mg.kg^{-1} and incubating for up to 52 weeks. A mean total of 35.8 % of the applied radioactivity was recovered as $^{14}CO_2$ within the test period. ^{14}C-brodifacoum was the major radiolabeled component in the soil extracts throughout the 52 weeks. Under the conditions of the study, the half-life of brodifacoum was calculated to be 157 days. A study was carried out with ^{14}C-bromadiolone in four types of soil. The rodenticide was degraded significantly with half-lives ranging from 1.8 to 7.4 days [38].

The study published by A s k h a m [1] was inspired by the crop use of currently registered rodenticides during the spring and late summer when rodents have been found to re-emerge and growers did not have any practical means of controlling their expanding population. Several aspects were investigated including the degree at which a commercially prepared rodenticide bait might withstand precipitations regimes and the subsequent potential crop contamination. In addition to other aspects, the translocation potential of bromadiolone from a growing medium to wheat was investigated. One hundred ml of a test solution containing 2199.39 pg ^{14}C-bromadiolone was applied to the soil surface of 12 growing wheat plants. An additional 100 ml of hydroponic solution (1389.03 pg ^{14}C-bromadiolone) was used for 12 wheat plants. The application of bromadiolone onto soil resulted in 353 ppb of bromadiolone in leaves and stems of wheat after 16 days while the level of bromadiolone in shoots grown in hydroponic solution was in the 2 to 3 ppm range, probably due to the lack of soil binding sites.

For public health reasons, ARs are applied in public infrastructures and sewage systems, especially in large urban areas where rodents can obtain food and shelter. They are released to sewage waters and reach wastewater treatment plants (WWTPs) through solubilisation of AR baits when applied to eliminate or control rodents in sewage systems, water channels, or due to agricultural runoff [23]. G ó m e z - C a n e l a et al. [22] tested the hypothesis that WWTP effluents can be a source of anticoagulants to receiving waters and that these can affect aquatic organisms and other non-target species. In their study, they determined the occurrence of 11 anticoagulants in WWTPs receiving urban and agricultural wastewaters. Warfarin was the most ubiquitous compound detected in influent waters and was partially eliminated during the activated sludge treatment, and low nanograms per liter concentration were found in the effluents. Other detected compounds were: coumatetralyl, ferulenol, acenocoumarol, flocoumafen, brodifacoum, bromadiolone, and difenacoum at concentrations of 0.86—87.0 ng.l^{-1}. Considering the water volumes of each WWTP, daily emissions were estimated to be 0.02 to 21.8 g.day^{-1}, which means that WWTPs contribute to the loads of anticoagulants to the receiving waters.

However, there is another important risk related to the discharge of anticoagulants to wastewaters. The products of aerobic biological treatment of urban wastewater (sewage) are treated water and sewage sludge. Because sewage sludge contains macronutrients (nitrogen and phosphorus), micronutrients (iron, zinc, copper, ...) and a high percentage of organic matter, it has valuable agronomic properties and is applied as fertilizer in many countries. The use of sewage sludge must take into account the nutrient needs of plants but should not compromise the quality of soil or surface water and groundwater [23]. G ó m e z - C a n e l a and L a c o r t e [23] developed a multiresidue method based on the soil-liquid extraction in combination with an optimized cleanup and liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the determination of ARs in sludge and used this method to evaluate the presence of ARs in 27 centrifuged WWTP sludge intended to be used as agricultural fertilizer. Bromadiolone was detected in sludge of six out of 27 WWTPs at concentrations between 4.77 and 7.73 ng.g^{-1}. This rodenticide has a low solubility which explains its accumulation in activated sludge with respect to the other ARs. Brodifacoum, the most toxic of the 2nd generation ARs was only detected in two of the 27 WWTP at concentrations of 15.2 and 17.4 ng.g^{-1}. The authors calculated the mass loads of ARs on a monthly basis considering the ΣAR concentration and the sludge generated in each WWTP. The minimum and maximum ΣAR loads were 0.04 and 65.8 g per month, respectively. Taking into account the mass loads generated by the WWTP every year, about 1.9 kg of anticoagulants are estimated to be eventually discharged to agricultural soils in north-east Spain. Their presence may trigger the accumulation of ARs in animals that prowl landfills and farmlands.

CONCLUSIONS

The results obtained in our study, investigating wheat grown on bait-contaminated soil, proved the presence of residues of both rodenticides, brodifacoum and bromadiolone in wheat exceeding considerably the ADI for these ARs. This indicates that despite the low solubility of both tested anticoagulant rodenticides, the field crops grown on soil exposed to the relevant baits may present considerable loads on the food chain. The acceptable daily intake was significantly exceeded in all the cases in dependence on the soil-incorporated bait dose. With brodifacoum and the 100 g.m^{-2} dose, the ADI was exceeded more than 700-fold and with the 500 g.m^{-2} dose even more than 1400-fold.

With bromadiolone and the 100 g.m^{-2} dose the ADI was exceeded 150-fold and after incorporation of 500 g.m^{-2} of the soil, the ADI in wheat was exceeded more than 350-fold. With regard to the recent studies that found the presence of rodenticide residues in water treated by WWTP and in sewage sludge that may be used in agriculture for irrigation or manuring, our results indicate the need for additional investigation of potential translocation of anticoagulant rodenticides to crops and the risks to their consumers.

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