



State of the art, gaps and future perspectives on common kestrel ecotoxicology

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ABSTRACT

Anthropogenic activities have caused a steady decline of common kestrel (*Falco tinnunculus*) since the 1980 s. Effects, especially sublethal effects of contaminants, need to be investigated to ensure the conservation of this species. Data about countries, biological material, contaminants classes, and methodological approaches were collected from scientific publications to highlight gaps on common kestrel toxicology and ecotoxicology. We found that most studies have been conducted in Europe and in the field, underlining a lack of in vitro studies. The studies investigated mainly contaminant levels, while sublethal effects, evaluation of emerging contaminants and use of non-invasive or low-invasive samples were scarce. This work shows important gaps on toxicological status of the common kestrel, highlighting the importance of developing a non-lethal approach that combines responses at different levels of biological organization, as well as data on chemical contamination and on the environment in which the different populations inhabit.

1. Introduction

The common kestrel (*Falco tinnunculus*), also known as the Eurasian kestrel, is a small and diurnal raptor of the *Falconidae* family. It is distributed in Europe, Africa and, Asia (Cardozo et al., 2016), spreading across open lands such as grassland, shrubland, farmland (Casagrande et al., 2008) and urban centres (Charter et al., 2007). The *Falco tinnunculus* is considered a partially migratory species because it's totally migrant in cooler northern Europe and more resident in central and southern regions (Baltag et al., 2014; Holte et al., 2016). This species is characterized by a strong sexual dimorphism (Zampiga et al., 2008) with evident plumage and size differences (Fargallo et al., 2002; Piauxt et al., 2012). Generally, it's a solitary species but can be gregarious in favourable habitat (Ermolaev, 2016). Its nests are located in rock crevices, cliffs, buildings, and occasionally, in abandoned nests of other bird species; this species also exploits nest boxes (Anushiravani and Sepehri Roshan, 2017). In Europe, eggs laying occurs between mid-March and early June (Costantini and Dell'Omo, 2020), the incubation lasts about one month and the ledging period varies from 27 to 39 days (Kabeer et al., 2021). The common kestrel is a predator with a varied and variable diet, depending on the geographic region and, therefore, on the type of prey availability. For example, it feeds mainly on small mammals in northern Europe, such as voles and harvest mice (Laaksonen et al.,

2008); otherwise, in southern regions, its diet is characterized by lizards and insects (Carrillo et al., 2017).

The global common kestrel population is around 4000,000 - 6500,000 mature specimens (BirdLife International, 2021) and about 19% of these individuals inhabit Europe (BirdLife International, 2016). The IUCN consider this species as "least concern" at the global level, although it is subject to moderate but steady decline since the 1980 s (BirdLife International, 2016). The European population size is estimated to be decreasing by about 25% in three generations (BirdLife International, 2015) and in some African countries the common kestrel population decreased by about 75–94% in the late 1960 s and early 2000 s (Thiollay, 2007).

The causes of the *Falco tinnunculus* decline are complex and multiple and are due to anthropogenic activities: agricultural intensification, pesticide use, habitat modification (e.g. sealed surfaces) (Grande et al., 2018), landscape simplification (e.g., proportion of arable land) (Butet et al., 2022), loss of nesting sites (Costantini and Dell'Omo, 2020), and even wind energy development (Strix, 2012). Among the different threats, the agricultural sector is the most important for the common kestrel decline (BirdLife International, 2016; Costantini et al., 2014). The plant protection products (e.g., insecticides, rodenticides, fungicides, and herbicides), widely used in this sector, can cause, for instance, alterations of nestling's body condition (Martínez-Padilla et al., 2017)

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and breeding success (Buck et al., 2020). Moreover, the effects of these substances can even be lethal (Hughes et al., 2013; Ruiz-Suárez et al., 2015). As an apex predator species, the common kestrel can accumulate higher concentration of contaminants than species at the lowest levels of the food web because of biomagnification processes (Yu et al., 2013).

In the last ten years, research on common kestrels has focused on contaminant accumulation (Jin et al., 2016; S. Kim et al., 2016; Luzardo et al., 2014; Roos et al., 2021), abundance (Butet et al., 2010; Gil-Tena et al., 2015; Teillard et al., 2014), behaviour (Abbasi et al., 2015; Schabacker et al., 2014) and reproductive performances (Costantini et al., 2014) in urban and agricultural habitats impacted by anthropogenic activities. At the same time, sublethal effects of toxic compounds have been poorly investigated (Bang et al., 2019; Buck et al., 2020; Shimshoni et al., 2012; Strong et al., 2015; Sumasgutner et al., 2018; Wemer et al., 2021).

The main aim of this paper was to review the available studies concerning the toxicology and ecotoxicology of the common kestrel to highlight any gaps and establish priorities for future investigation. In addition, the specific goals were to evaluate where the studies were conducted, the type of contaminants investigated, the considered life stages, the biological material tested, and the methodological approaches used.

2. Material and methods

A systematic quantitative approach (Pickering and Byrne, 2014), with some modifications, was adopted to conduct the research on common kestrel toxicology. Electronic bibliographic databases research was conducted on Scopus and Google Scholar and a time limit of eleven years (from 2010 until 2021) was adopted for the research. A group of keywords was established to identify relevant studies, where “*Falco tinnunculus*” was combined with the following terms: “toxic”, “toxicology”, “contaminants”, “pesticides”, “heavy metals”, “sublethal effects” and “biomarkers”. Only articles in English language and peer-reviewed were collected, while reviews were not included, although their references were checked to guarantee that no publications were missed. The references of selected articles and papers with one or more cited articles on *F. tinnunculus* were screened to assess whether they contained relevant studies to include in the present review. Book chapters, conference abstracts, reports, and other types of grey literature were not considered for this work.

Our search resulted in a total of 1226 studies, combining all the keywords and setting the parameters listed above. Articles regarding ecology, physiology, immunology, parasitology, toxicity of endogenous compounds and causes of admission to rescue centres were not selected, as long as the evaluated parameters were not correlated with contaminant exposure. Studies on physiological, biochemical/cellular, and behavioural variations in common kestrel of urban and agricultural areas variously exploited by human were included. The final selection comprised a total of 58 articles that met our criteria. The selected papers were examined in detail, recording the following information in an Excel database: year of publication, geographical location, life stage, sex, study type, biological material, contaminant classes, methodological approach and results of the paper.

If a work included multiple categories for any variable, all were considered in the final analyses and the definition “not specified” was assigned when a category information was missing.

The variable “studies type” was divided into four categories: “historical collection”, “historical database”, “field studies” and “laboratory studies”. “Historical collection” and “historical database” indicated respectively the studies performed on biological material (such as feathers) kept in museums and datasets on poisoning events. “Field studies” included works conducted on wild birds, and “laboratory studies” identified exposure experiments in the laboratory. The “life stage” was divided into four categories: “eggs”, “nestlings”, “juveniles” and “adults”. The variable “methodological approach” was divided into

the following categories: accumulation, biochemical/cellular responses, dietary habits, body condition, behaviour, parasitology, poisoning, reproductive success and risk assessment. In particular, “accumulation” and “poisoning” papers evaluated the contaminant levels and poisoning events respectively. Biochemical/cellular responses” and “parasitology” studies included the molecular research and parasites presence, respectively. “Body condition” indicated the studies that calculated the body condition index and “reproductive success”; the latter referred to research on, for example, clutch size and hatching success. “Behaviour” and “dietary habits” described studies on this species abundance in a specific area and on dietary sources and trophic position. Concerning “risk assessment” research that evaluated, for instance, the Toxicity Exposure Ratio (TER) or the Hazard Quotient (HQ) were identified.

The results of each study were categorized with a symbol “+” when a study showed an effect and the symbol “-” when the authors reported no effect. For each study, the sample size, the result range or the mean value with standard deviation and the corresponding unit of measurement were recorded.

3. Results and discussion

The 58 studies selected on toxicology and ecotoxicology of the common kestrel, obtained from the bibliographical research spanning from 2010 to 2021, are reported below and divided by the recorded information: year of publication; geographical location; life stage and sex; study type; biological material; contaminant classes; methodological approach and results of the paper. Most of these studies (44) were conducted on multiple avian species, and only 14 considered just *F. tinnunculus*. The multispecies approach is often adopted to evaluate the levels or effects of different contaminants on avian species, which may have different feeding habits (diurnal or nocturnal), diet composition (e.g., insectivorous or carnivorous), or behaviour (e.g., breeder or resident) (Ma et al., 2021).

3.1. When and where the studies were performed

During the last eleven years, 58 papers were published with an average of five per year, and the major number of studies was published in 2014 (7) and 2021 (9) (Fig. 1).

The studies were conducted in all the countries where the species is distributed, in Eurasia and Africa, (Village, 1990). Specifically, most of the studies were carried out in Europe (40), followed by Asia (16) and Africa (2). The number of studies conducted in Europe can be related to the large number of European monitoring programmes that used birds of prey to investigate the impact of contaminants on the environment, were the common kestrel was one of the diurnal raptors most used as bio-indicator (Gómez-Ramírez et al., 2014). Moreover, the high number of articles published by European researchers is also probably due to common kestrel population decline events (e.g., between 1980 and 2016) mainly related to agricultural practices (Roos et al., 2021).

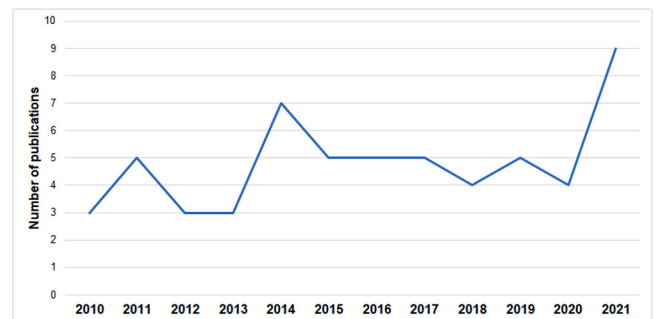


Fig. 1. Number of studies on common kestrel toxicology and ecotoxicology that met the criteria for inclusion in this review divided by year of publication.

Indeed, as reported by the [European Environment Agency \(2020\)](#), Europe is one of the continents with the most intensive agriculture and forestry activities. To confirm this, among the European countries, Spain (12) and France (6) were the ones with the highest number of publications. According to [Eurostat \(2021\)](#) these countries are part of the “big four” that strongly contribute to the EU’s agricultural industry. It is maybe for this reason that in Spain and France, there was a greater interest in the evaluation of pesticides effects and habitat simplification on avian species such as common kestrel ([Bouvier et al., 2011](#); [Buck et al., 2020](#); [Butet et al., 2010](#); [Filippi-Codaccioni et al., 2010](#); [Gil-Tena et al., 2015](#); [Luzardo et al., 2014](#); [Martínez-Padilla et al., 2017](#); [Rial-Berriel et al., 2020, 2021](#); [Ruiz-Suárez et al., 2014, 2015](#); [Teillard et al., 2014](#); [Valverde et al., 2020](#)). On the other hand, despite Italy and Germany are included in the “big four,” the number of publications in these countries was low: two and one, respectively. Four studies were published in United Kingdom (UK), probably because of the significant decline (–35%) that the Eurasian kestrel suffered in this country between 1995 and 2018 ([Harris et al., 2020](#)).

In general, however, the number of articles published by European researchers is still low considering the population decrease shown in many European countries, as published by Bird International (2021).

Regarding the Asian continent, most papers were conducted in Korea (6) and China (5). [Abbasi et al. \(2016\)](#) have reported a study’s scarcity on the contaminant accumulation and toxicity of Asian birds. This finding, together with the growing and rapid industrialization of this continent, has prompted researchers to investigate accumulation and contaminant effects ([Bang et al., 2019](#); [Barghi et al., 2018](#); [Jin et al., 2016](#); [Kim and Oh, 2016](#); [Kim et al., 2016](#); [Ma et al., 2021](#); [Nam and Lee, 2011](#); [Shimshoni et al., 2012](#); [Yin et al., 2018](#); [Yu et al., 2013](#); [Yu et al., 2011](#); [Zhang et al., 2021](#)).

The only two countries to have carried out studies on common kestrel toxicology in the African continent were Nigeria (1) and Egypt (1). The two papers were published recently, in 2021 and 2019 respectively. The only study evaluating the biopesticide effects in the last ten years was conducted on Nigerian common kestrel ([Mullié et al., 2021](#)).

This first analysis reveals that the research numbers at the global level are still too poor to guarantee a good monitoring and conservation of common kestrel.

3.2. Study type

Most of the studies of this review were conducted in the field (54), while research carried out on historical collections (1), historical databases (2), and in the laboratory (2) were only a few.

The “historical collection” research is an approach that allows to assess the geographical and temporal trend of the bird exposure to contaminants. Another positive aspect of this type of study is that the retrospective reference values obtained can be compared with current data of the same species living in the same geographical area ([Movalli et al., 2017](#)). Similarly, the studies on “historical databases” of poisoning can also be used for geographical and temporal trend evaluations and for identifying any flaws in the monitoring procedures related to toxic events. For example, [Gil-Sánchez and collaborators \(2021\)](#) showed that many animal groups, including the common kestrel, are over-represented in the national poisoning database while others are underrepresented. In general, this type of study offers many advantages even though such studies are still limited.

Regarding the “laboratory study,” the number of publications was very low, probably because the common kestrel is strictly protected by different international agreements, limiting its use for scientific purposes.

[Valverde and collaborators \(2020\)](#) conducted an in vivo experiment on kestrel specimens recovered in a rescue center in southeastern Spain. The birds were exposed to bromadiolone, an anticoagulant rodenticide, to investigate its persistence at different carcass decomposition stages. It

was possible to use these specimens for the experiments because they were intended for euthanasia due to severe wing injuries and all procedures complied the ethical standards. Their results suggest that the rodenticide persists until the first weeks after death under certain weather conditions; then the carcasses can be a source of wildlife poisoning. *In vivo* experiments are considered a valid approach for evaluating the contaminant effects, although they have obvious limitation due to the protected status of Eurasian kestrel and particularly for ethical issues. Therefore, in vivo testing to evaluate the contaminant effects is generally not desirable for different reasons. A valid alternative to in vivo studies could be in vitro research. This type of studies has many advantages such as animal-use reduction, control of chemical and physical environment, use of non or low-invasive matrices, and finally repeating experiment for several times. However, to this date, no study adopted an in vitro approach on common kestrel. Indeed, this type of approach is applied on other birds, reptiles and marine mammal species, showing to be a valuable tool to unravel interactions between contaminants and organisms and eventually also establish contaminant dose-response relationships ([Bianchi et al., 2022](#); [Crump et al., 2008](#); [Hernández-García et al., 2014](#); [Leena Mol et al., 2012](#); [Oropesa et al., 2013](#)). Moreover, in some studies, a good correlation between in vivo and in vitro experiments was shown ([Billiard et al., 2004](#); [Head and Kennedy, 2010](#); [Stadnicka-Michalak et al., 2015](#)).

3.3. Life stages and sex

Concerning the life stages, fifteen studies (15) were conducted on adults, six (6) on nestlings, four (4) on eggs, and four (4) on juveniles. In thirty-seven (37) articles the specimen’s life stage was not indicated or was not referred to the specific results. Most studies (55) did not specify the specimen’s sex. Only 5 and 4 studies were conducted on males and females, respectively. In some articles of this research (8), age or sex were included as variables in regression models, even if only few works reported the specific result of the parameter measured related to stage of life or sex, as seen above.

In ornithology, it has been shown that the results of physiological parameters may differ depending on animal life stage and gender ([Hao et al., 2021](#)); it is therefore important to consider this aspect in ecotoxicological research too. For example, contaminant concentration can be higher in adult than juvenile birds due to the longer time exposure ([Fritsch et al., 2019](#)). [Zhang and collaborators \(2021\)](#) highlighted a higher contaminant (PCDD/Fs DL-PCBs and indicator of PCB) concentration in adult than juvenile common kestrels. On the contrary, the work of [Grúz and collaborators \(2019\)](#) did not show any significant differences between common kestrel age for the heavy metals (Cd, Cr, Pb and Hg) investigated. Concerning the sex variable, male and female specimens, having different hormonal levels, can show different responses in several parameters ([Tartu et al., 2014](#)). In this review only three papers evaluated differences between sex ([Grúz et al., 2019](#); [Hromada et al., 2011](#); [Zhang et al., 2021](#)). The results obtained by [Hromada and collaborators \(2011\)](#) on the Toxicity Exposure Ratio (TER) for rodenticides showed a difference between specimens’ gender with a lower risk of poisoning in females than males *F. tinnunculus*. Both [Zhang and collaborators \(2021\)](#) and [Grúz et al. \(2019\)](#) showed no difference in the levels of contaminants between specimens of different sex. Taking into account the above results, life stage and sex in ecotoxicological research on birds remains an important point to be considered.

3.4. Biological materials

In the last ten years, as shown in [Fig. 2a](#), 42 studies have been conducted on non-invasive or low-invasive samples of Eurasian kestrel: blood (6), eggs (5), feathers (10) and whole organism (21). The category “organism/population” includes papers on risk assessment, reproductive success, behaviour, and body condition that consider the specimen or the population in their entirety. Other 42 studies were conducted on

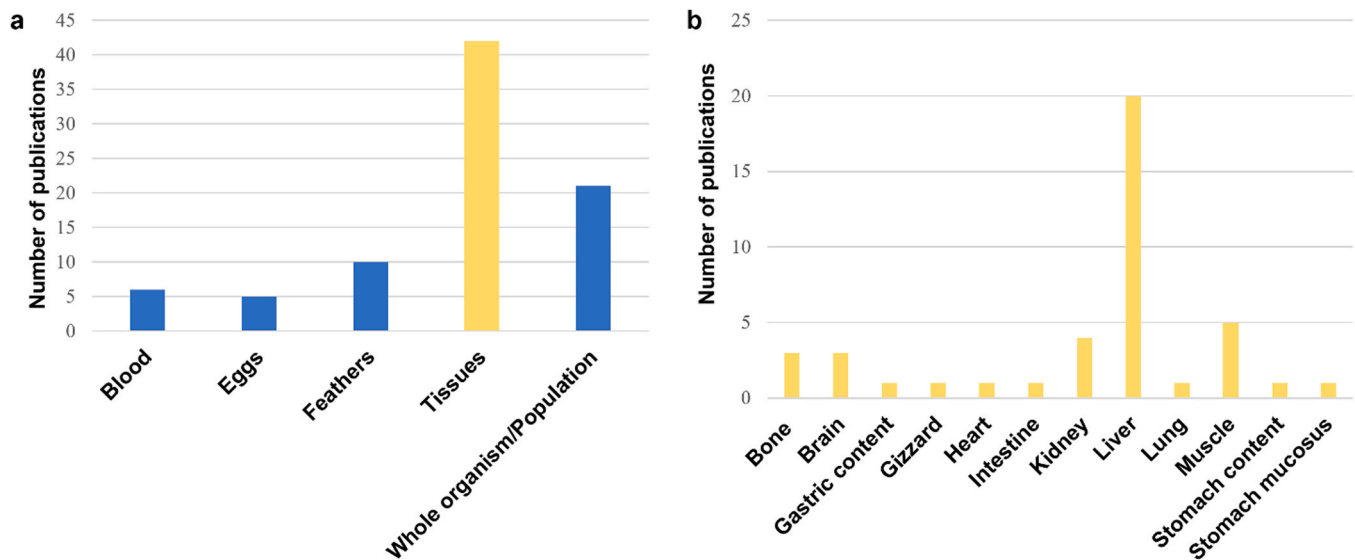


Fig. 2. Number of studies that met the criteria for inclusion in this review, divided by biological material (a) and the specific tissues used (b).

tissues obtained with a destructive approach (Fig. 2b): liver (20), muscle (5), kidney (4), bone (3) brain (3) and gastric content (1), gizzard (1), heart (1), intestine (1), lung (1), stomach content (1) and mucosus stomach (1). Of these, 7 papers have used more than one tissue.

The investigations that used a destructive sampling of the animals are carried out on stranded birds or birds to be euthanized in the rescue centers and provide good results on the degree of contamination of the animals (García-Fernández et al., 2013). However ethical and legal limitations are present and must be underlined (Yin et al., 2018). In addition, the use of samples from stranded or euthanized animals does not always guarantee a statistically significant number for an adequate assessment of the health status of the species. In the last years, there is a growing interest in the use of non-invasive (excreta, moulted feathers and eggshells) or low-invasive (blood and plucked feathers) biological materials. Among the various tissues available, most studies using a non-lethal approach focus on feathers (8 out of 10) because they are easy to collect and create a very low stress level on the animals. The feathers are also a valuable tissue for studying the effects of contamination by

heavy metals, other non-essential elements (Manjula et al., 2015) and legacy POPs (together with plasma and preen oil). However, a Norwegian study showed that feathers are not suitable for the analysis of emerging contaminants (Løseth et al., 2019). Some authors (Abbasi et al., 2017; Manzano et al., 2021) also used this biological material to investigate the feeding ecology by stable isotopes analysis ($\delta^{13}C$ and $\delta^{15}N$). In recent years the use of blood has increased to study the ecotoxicological effects of different contaminants on common kestrel (Berny et al., 2017; Martínez-Padilla et al., 2017; Rial-Berriel et al., 2020; Valverde et al., 2020; Wemer et al., 2021). Although it has proven to be an efficient material for the assessment of sublethal effects (Espín et al., 2016), not only in birds but in other protected species, the number of research studies using blood remains low.

In conclusion, when dealing with endangered species, the use of non and low-invasive, rather than invasive or destructive techniques, is preferable, as suggested by several authors (Casini et al., 2018; Fossi and Leonzio, 1994; Hopkins et al., 2005).

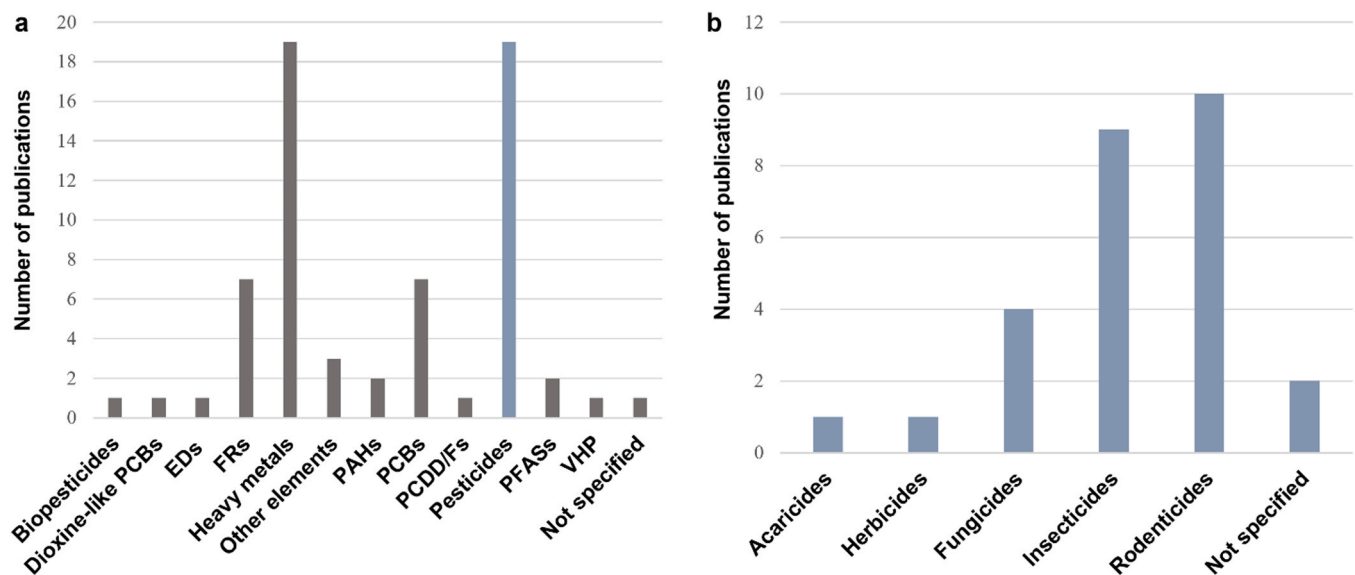


Fig. 3. Number of studies that met the criteria for inclusion in this review, divided by contaminant (a) and pesticide classes investigated (b). The “not specified” category referred to articles on poisoning without a contaminant investigation (a) or to papers without details on pesticide classes (b).

3.5. Contaminant classes

Regarding the classes of contaminants investigated in common kestrel, the Fig. 3a shows that most of the research studied the presence or the effects of pesticides (19) and heavy metals (18), followed by studies on flame retardant (7) and polychlorinated biphenyls (7). Among the 19 articles on pesticides, 10 focused on rodenticides class, followed by insecticides (9) and fungicides (5). The other types of pesticides were less investigated (Fig. 3b). In three of the 19 articles on pesticides, more than one class of pesticides were studied. In addition, eleven papers assessed effects or concentration of different substances belonging to the same class of pesticides. The most investigated chemical classes of pesticides were organophosphates (12), followed by coumarins (6), carbamates (5) and organochlorines (5). Regarding the studies on effects and abundance of heavy metals, the elements most investigated were Pb (13), Cd (12), Hg (9), Cu (8) and, Cr (6). The other heavy metals were less investigated (Appendix Table A1). Thirteen of the 19 papers on heavy metals measured more than one element. The large number of heavy metal studies is probably linked to the fact that birds, especially birds of prey, as top predators, tend to accumulate heavy metals (Abbasi et al., 2015), and they are then considered good bioindicators for the monitoring of toxic compounds (Grúz et al., 2019; Movalli et al., 2017; Espín et al., 2016).

Regarding pesticides, the strong interest in this topic is probably due to the fact that intensive agricultural activities require the increasing use of plant protection products (PPTs) for crops control. Since the 1970 s, it has emerged that many of these substances, some of which are no longer in use, have adverse effects on non-target species, including birds. Several authors studied the effects of PPTs on birds, suggesting that these compounds can accumulate in common kestrel (Bang et al., 2019; Barghi et al., 2018; Bouvier et al., 2011; Buck et al., 2020; Christensen et al., 2012; Hromada et al., 2011; Hughes et al., 2013; Kim et al., 2016; Luzardo et al., 2014 Martínez-Padilla et al., 2017; Rial-Berriel et al., 2020, 2021; Roos et al., 2021; Ruiz-Suárez et al., 2014, 2015; Schabacker et al., 2014; Shimshoni et al., 2012; Valverde et al., 2020; Yu et al., 2013). Sublethal effects, evaluated through acetylcholinesterase (AChE) (Bang et al., 2019; Shimshoni et al., 2012) and Protoporphyrin IX (Buck et al., 2020), reproductive success (Buck et al., 2020; Costantini et al., 2014; Sumasgutner et al., 2014, 2019), behaviour (Abbasi et al., 2015; Bouvier et al., 2011; Butet et al., 2010; Filippi-Codaccioni et al., 2010; Gil-Tena et al., 2015; Kitowski et al., 2016; Mullié et al., 2021; Roos et al., 2021; Schabacker et al., 2014; Sumasgutner et al., 2014, 2019; Teillard et al., 2014; Yaneva et al., 2020;), poisoning events (Gil-Sánchez et al., 2021; Hughes et al., 2013; Ruiz-Suárez et al., 2015) and risk assessment (Hromada et al., 2011; Yin et al., 2018; Yu et al., 2013; Zhang et al., 2021) were also investigated.

Various studies have also evaluated the effects of pesticides on common kestrel because some of these compounds, such as rodenticides and insecticides, have lethal effects on organisms that are the basis of the common kestrel diet. Feeding mainly of rodents, this species could suffer a secondary exposure to the substances mentioned above (Hughes et al., 2013). The reducing in the abundance of preys could also have an indirect effect by the generation of a series of cascading effects as diet modification, some food deficiency and, consequently, a reduction of the body condition, making the animal more susceptible to any pathologies.

Although fungicides and herbicides are among the most used pesticides in agriculture, to date they are poorly investigated in *F. tinnunculus*.

Few works monitored the emerging contaminants (ECs), such as perfluoroalkyl (PFASs) (Barghi et al., 2018; Eriksson et al., 2016), novel flame retardants (OPFRs, DPs, NBFs, DBDPE, BTBPE, EHTBB, BEHTBP) (Abbasi et al., 2017; Jin et al., 2016; L. Yu et al., 2013) and UV filters (Molins-Delgado et al., 2017). In addition, no studies have been found on bisphenol replacement compounds such as BPS, BPF, BPAF, and chlorinated paraffins (CPs). An increase in studies on ECs is necessary, given that these compounds are widely spread in different

environmental compartments (González -Rubio et al., 2021) and their effects on the common kestrel are poorly understood.

3.6. Method approaches

The majority of studies evaluated the accumulation of contaminants in common kestrel (40), followed by research on behaviour (13), dietary habits (7), biochemical/cellular responses (5), body condition (5), reproductive success (4) and risk assessment (4). The articles focused on parasitology (3) and poisoning (3) were lower (Fig. 4). Twenty-three articles of the 58 included in this review evaluated at least two different endpoints.

3.6.1. Accumulation

The common kestrel, as an apex predator, can accumulate different classes of contaminants; for this reason, the number of works on the accumulation was the highest (40 out of 58). The results of all works on accumulation included in the review are summarized in the appendix (Table A2). Twenty-four of the works evaluated exclusively this parameter, while 16 articles combined this information with parameters aiming to evaluate their possible effects on the species. Only 9 out of 24 investigated the presence of multiple classes of contaminants (Abbasi et al., 2017; Barghi et al., 2018; Buck et al., 2020; Jin et al., 2016; Luzardo et al., 2014; Molins-Delgado et al., 2017; Rial-Berriel et al., 2020; L. Yu et al., 2013; Zhang et al., 2021). These results show the lack of an integrated approach that combines contaminant data and sublethal effects. Studies that use an integrated approach would provide more comprehensive information that can be used for the conservation of this species. Unfortunately, only a few authors adopted this method, for example Buck and collaborators (2020) evaluated OCs and PCBs levels, the effects on reproductive success (eggshell thickness), and the protoporphyrins IX concentration as a biochemical response in eggs. Similarly, Bang et al. (2019) also evaluated the accumulation of OP pesticides and sublethal effects by a biomarker test (AChE activity). However, the evaluation of several toxicological responses would have been desirable in order to obtain a more complete view of the studied contaminant effects.

In addition, most accumulation studies were conducted on destructive material (26 out of 40), such as liver, muscle, kidney, brain, etc. However, accumulation analysis can also be conducted on non-invasive or low-invasive material like excreta, blood and feathers. These tissues also enable the evaluation of the possible effects of toxic substances at different levels of biological organization.

3.6.2. Biochemical and cellular responses

Among the papers that investigate the biochemical and cellular responses (5 articles), AChE activity (2), protoporphyrin IX (1) and carotenoid coloration (1) were assessed. One paper only evaluated more than one parameter (complement system activity; haptoglobin concentration, Hp; natural antibodies activity, Nab; total glutathione, tGSH; ratio glutathione:glutathione disulphide, GSH:GSSG) (Table 1). Both the studies on AChE highlighted basal values of the specimen ($8-12 \pm 1.77$ $\mu\text{mol}/\text{min}/\text{g}$), but the activity values of exposed animals are not showed or determined. Buck and collaborators (2020) evaluated the concentration of protoporphyrins IX (a reddish-brown pigment in avian eggshells) as a biomarker of exposure to OCs showing a protoporphyrins concentration reduction with the increasing hexachlorobenzene level in the egg content. Although many OCs have been banned since the 1970 s, the results of this paper suggest continuing to monitor the presence of these substances in the environment in order to assess the effects of regulatory actions. The effects of urbanisation on common kestrel nestlings were also investigated by biochemical (Wemer et al., 2021) methods and skin yellowness measurements (Sumasgutner et al., 2018). Wemer et al. (2021) found that kestrel nestlings present in more urbanized areas had lower hemolysis, while none of the other immunotoxicity (Nab and Hp concentration) and oxidative stress (tGSH and

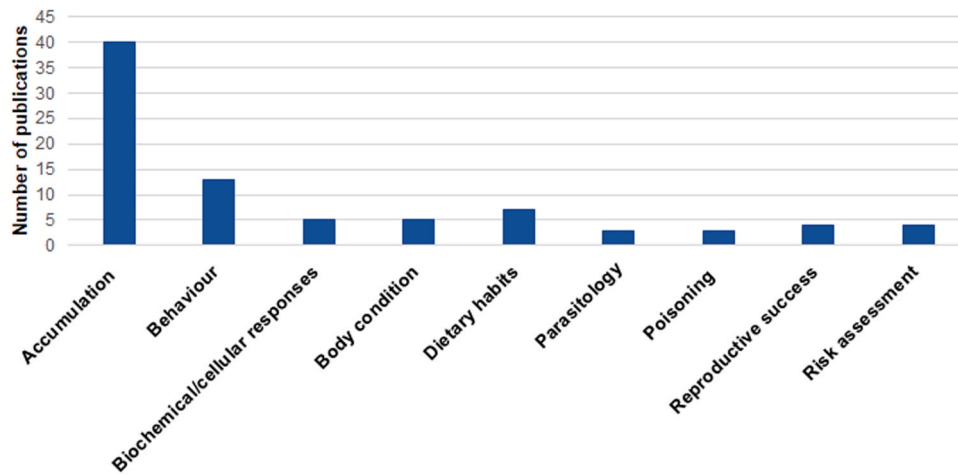


Fig. 4. Number of studies that met the criteria for inclusion in this review, divided by methodological approaches adopted.

Table 1

Studies that met the criteria for inclusion in this review, divided by biochemical/cellular parameters evaluated. The indicated studies did not specify the animal's sex, and all were conducted in the field. The abbreviation ns means "not specified", the symbols "+" and "-" indicate an effect or no effect, respectively. nd indicates "not detected" and asterisk (*) refers to indicative results extrapolated from the graphs.

Life stage	Biological material	Contaminant	Human exploited environments	Methodological approach	Sample size	Results (mean ±sd; mean; min-max)	Unit of Measurement	Reference, year
Nestlings	Blood	ns	Urban	tGSH	143	-	ns	Wemer et al. (2021)
				GSH:GSSG	69	-		
				Complement system activity	69	+ ns		
				NAb activity [Hp]	69	-		
Eggs ns	Egg Brain	HCB No pesticide exposure	Urban	Protoporphyrin IX	40	+ 0-120 *	nmol/g d.w. µmol/min/g tissue	Buck et al. (2020) Bang et al. (2019)
				AChE activity	5	8.66 ± 1.04		
Nestlings ns	Animal Brain	ns Insecticide No pesticide exposure	Urban	Carotenoid coloration	154	+ -0.6 - 0.4 *	µmol/min/g tissue	Sumasgutner et al. (2018) Shimshoni et al. (2012)
				AChE activity	21	- nd 12.4 ± 2.5		

Table 2

Studies that met the criteria for inclusion in this review, divided by dietary habits parameters evaluated. The animal's sex was not specified, and all the indicated studies were conducted in the field. The abbreviation ns means "not specified", the symbols "+" and "-" indicate an effect or no effect, respectively.

Life stage	Biological material	Contaminant	Methodological approach	Sample size	Results (mean±sd; mean; min-max)	Unit of measurement	Reference, year
Adults	Feathers	Heavy metals and other elements	Dietary source (δ13C)	37	-24.06 - 19.29	%	Manzano et al. (2021)
			Trophic position (δ15N)	37	+ 6.27-15.25		
Adults	Liver	PCBs, DDTs, CHLs, CBz and PFASs	Trophic position (δ15N)	4	+ ns	%	Barghi et al. (2018)
ns	Feathers	PBDEs, HBCDDs, BTBPE, BEHTBP and EH-TBB	Dietary source (δ13C)	4	- -20.4 ± 4.8	%	Abbasi et al. (2017)
Eggs	Egg	BP1, BP3, 4HB, 4 DHB, ODPABA, OC and UVP	Trophic position (δ15N)	10	- + 8.3 ± 2.1	%	Molins-Delgado et al. (2017)
			Trophic position (δ15N)	10	- 8.58-11.45		
Eggs	Egg	PFASs	Dietary source (δ13C)	40	-28.2	%	Eriksson et al. (2016)
			Trophic position (δ15N)	40	5.3		
Adults	Liver	PBDEs, BEHTBP, DBDPE, BTBPE and DP	Trophic position (δ15N)	4	+ 7.0-9.4	%	Jin et al. (2016)
ns	Muscle	PBDEs	Dietary source (δ13C)	23	-18.4 ± 1.7	%	Yu et al. (2011)
			Trophic position (δ15N)	23	7.2 ± 1.2		

GSH:GSSG) parameters were altered. Sumasgutner and collaborators (2018) showed a reduction in carotenoid coloration in nestlings located in the city centre of Vienna. Further studies are needed to better enhance the relation between landscape ecology and biochemical and cellular responses. From this review, it emerged that biomarkers are scarcely used as predictive methodology of common kestrel exposure to contaminants. Further studies should also include the use of a set of biomarkers capable of providing different toxicological responses, such as neurotoxicity, oxidative stress, immunotoxicity, and genotoxicity.

3.6.3. Dietary habits

Seven articles investigated the common kestrel dietary habits related to contaminants exposure (Table 2). These aspects are usually examined by stable isotope analyses of carbon and nitrogen ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) because they permit to define organisms' dietary sources and trophic position (Yu et al., 2011) and their relation with the contamination (Abbasi et al., 2017). In particular, $\delta^{13}\text{C}$ reflects the carbon source, while the $\delta^{15}\text{N}$ can indicate the trophic level because its values enrich by 2–5‰ per trophic level (Eriksson et al., 2016). These parameters permit to investigate how the diet can increase or influence the exposure, accumulation, and biomagnification of a given contaminant (Jin et al., 2016; Molins-Delgado et al., 2017). This analysis can be carried out on different tissues (such as feathers, eggs, blood), but different information is obtained according to the used tissue. The isotopic composition of unhatched eggs reflects the isotopic signature of the mother birds (Molins-Delgado et al., 2017); the composition of feathers represents the diet habits during the moulting period (Manzano et al., 2021), whereas blood components reflect the trophic strategy during days or weeks before blood collection (Cherel et al., 2014; Kurle et al., 2013).

3.6.4. Body condition

A total of 5 papers evaluated the common kestrel body condition (Costantini et al., 2014; Martínez-Padilla et al., 2017; Sumasgutner et al., 2018, 2019; Wemer et al., 2021) (Table A3 in appendix). Wemer et al. (2021) found a negative correlation between body condition and urban gradient, and they assumed that the lack of suitable prey determined this condition. On the contrary, Sumasgutner and collaborators (2018) did not identify any relation between body condition and the urban environment. Regarding agricultural environments, nestlings from intensively cultivated areas showed worse body condition than those from grassland lands (Costantini et al., 2014). Sumasgutner et al. (2019) found no variation in the common kestrel adults from more or less exploited agricultural areas. Body condition was low in nestlings from two areas from central of Spain with residues of the rodenticide Bromadiolone (Martínez-Padilla et al., 2017). The body condition is

considered an indicator of individual's health or quality (Peig and Green, 2009; Labocha et al., 2014). It is routinely used in avian studies and can include different morphological and physiological metrics that represent the nutritional, immune, and hormonal state of a specimen (Frauendorf et al., 2021). One of the most used body condition index is the body mass of an individual corrected to its size or age, and it is commonly applied to quantify energy reserves in birds (Costantini et al., 2009). The articles included in this review evaluated the body condition using morphological metrics and applying validated methods (García-Berthou, 2001; Green, 2001; Roulin et al., 2007). Considering all the results obtained from the above studies, it would be interesting to proceed with other studies that evaluate the relationship among physiological parameters, levels of contaminants and body condition index.

3.6.5. Behaviour

Eight (8) of the 13 studies on behaviour (Table 3) examined the common kestrel abundance and the occurrence frequency, four (4) investigated the feeding behaviours, and one (1) the timing of breeding. The studies included in this review show that habitat simplification and the potential presence of environmental contaminants can affect the common kestrel behaviour. From a research carried out in the UK (Roos et al., 2021), which combined historical data on Second Generation Anticoagulant Rodenticides (SGARs) levels with data on kestrel abundance (from 1997 to 2012), emerged that its presence decreased with the rodenticide's concentration increase in the same considered year. Gil-Tena et al. (2015) showed that the composition of the agricultural landscape could influence farmland bird diversity. This study displayed that the common kestrel, preferring areas with shrubs and hedges, was less present in more impacted agricultural areas, such as those cultivated with cereals, rotational grassland, or artificial lands. Similarly, Butet et al. (2010) showed a decrease, although not significant, in the abundance of the kestrel in western France, where grasslands, hedgerows, and wooded area decline are present. Another author highlighted that the kestrel could be exposed to pesticides even in non-agricultural grassland scenarios, such as golf courses, due to its high frequency in this type of landscape (Schabacker et al., 2014). The urban environment, like agriculture one, can also cause behavioural changes on birds (Møller and Ibáñez-Álamo, 2012), including common kestrel. The urban environment facilitates the permanence of the species as there is a greater availability of nesting sites; this may represent an ecological trap, as the species is induced to eat small birds instead of rodents, modifying its diet (Sumasgutner et al., 2014). Mullié et al. (2021) found that individuals who ate grasshoppers treated with biopesticides (used to deal with entomopathogenic infestation by *Metarhizium acridum*) were subjected to a variation in food choice, preferring larger female locusts over smaller male ones. Up to date, no studies integrated behavioural results with

Table 3

Studies that met the criteria for inclusion in this review, divided by behavioural parameters evaluated. The animal's sex was not specified, and all the indicated studies were conducted in the field. The abbreviation ns means "not specified", the symbols "+" and "-" indicate an effect or no effect, respectively.

Life stage	Biological material	Contaminant	Human exploited environments	Methodological approach	Sample size	Results	Unit of measurement	Reference, year
ns	Animal	<i>Metarhizium acridum</i>		Feeding behaviour	40	+ ns		Mullié et al. 2021
ns	Animal	Rodenticide ns		Abundance		+ ns		Roos et al. (2021)
ns	Animal		Agricultural	Abundance		-		Yaneva et al. (2020)
Adults	Animal			Timing of breeding	448	-		Sumasgutner et al. (2019)
Adults	Animal	Heavy metals		Feeding behaviour	3	-		Kitowski et al. (2016)
ns	Animal	Heavy metals		Feeding behaviour	5	+ ns		Abbasi et al. (2015)
ns	Animal		Agricultural	Abundance		+ ns		Gil-Tena et al. (2015)
ns	Animal	Pesticide ns	Non-agricultural (golf course)	Frequency of occurrence		+ 55	%	Schabacker et al. (2014)
Nestlings	Animal		Urban	Feeding behaviour	763 Nests	+ ns		Sumasgutner et al. (2014)
ns	Animal		Agricultural	Abundance		-		Teillard et al. (2014)
ns	Animal	Insecticides ns Fungicides ns		Frequency of occurrence		+ 20	%	Bouvier et al. (2011)
ns	Animal		Agricultural	Abundance		+ ns		Butet et al. (2010)
ns	Animal		Agricultural	Abundance		-		Filippi-Codaccioni et al. (2010)

molecular, biochemical, cellular and physiological investigations to obtain information on the general health status of the species. Indeed, further studies that investigate effects at different biological levels of organization are needed for the conservation of the species.

3.6.6. Parasitology

The presence of parasites was evaluated in three studies of the review (Table A5 of appendix). Wemer et al. (2021) and Sumasgutner et al. (2018) conducted their research on kestrel nestlings from the city of Vienna. Both studies did not show any infection of ectoparasite *Carnus hemapterus* related to urban gradient. However, ectoparasites were higher in junior siblings than their senior siblings (Wemer et al., 2021) and in earlier nests of the season (Sumasgutner et al., 2018). A result similar to the latter was obtained again by Sumasgutner and collaborators (2014), where a seasonal decline of parasite infection intensity in kestrel chicks was found. Sumasgutner and collaborators (2018) also showed that parasite infection was higher in chicks with less intense skin yellowness, probably because the immune system is activated to fight the parasite infection. Therefore, it requires more circulating carotenoids that cannot be used for pigmentation.

On the contrary, Sumasgutner et al. (2019) evaluated a blood parasite infection (*Haemoproteus*, *Plasmodium*, and *Leucocytozoon*) of a common kestrel migratory population from agricultural fields in western Finland. The parasite infection was used as a parameter of individual quality (together with body condition). It was not correlated with landscape homogenization linked to agricultural intensification.

As shown, there are very few studies on parasitology related to urban and rural gradients and research that investigate the interplay between the type of environment, level of contaminants, and individual quality. In addition, more studies on the cause of the parasite's presence are necessary to understand all interactions with physiological signals (i.e., immunotoxicity and oxidative stress biomarkers).

3.6.7. Poisoning

Poisoning events were evaluated in three articles (Table a4 in appendix). Gil-Sánchez et al. (2021) compared the results of their field observation with the national poisoning database (from 1990 to 2015) of Spain. They showed an over-representation of the common kestrel in the intentionally poisoned database. Ruiz-Suárez and collaborators (2015) found that in the Canary Islands, the intentional poisoning of the common kestrel was still frequent from 2010 to 2013. In fact, out of 17 kestrels analysed, pesticides poisoned 15 specimens. These results show the relevance of pesticide poisoning as cause of death of common

kestrels from the Canary Islands. It is important to note that among the pesticides found in carcasses, two (Carbofuran and Aldicarb) were already banned in the EU during the years of research. Finally, Hughes et al. (2013) highlighted that up to 23 kestrels, 41% were exposed to rodenticides. These results contrast with the Wildlife Incident Investigation Scheme (WIIS) Scotland, where among the causes of death of kestrels poisoning was 0%. From these results it emerged that this kind of study is still helpful for wildlife conservation.

3.6.8. Reproductive success

Most of the parameters investigated on reproductive success were fledging success (4) and hatching success (3), followed by clutch size (2), number of fledglings (2), nestling survival (1), egg volume (1), and eggshell thickness (1) (Table 4). The majority of these studies highlighted that urban and agricultural environments could affect the breeding success of common kestrel. The nests failure of urban kestrels in the city of Vienna was caused by predation or desertion of the nests and it occurred during eggs incubation. Moreover, male chicks were more subjected to death, and eggs hatched later than the average of the specie (Sumasgutner et al., 2014). Costantini and collaborators (2014) recorded a delayed egg-laying and a poor condition of offspring in specimens from the Italian intensive agriculture areas, and no effects on reproductive parameters. On the contrary, Sumasgutner et al. (2019) showed that the landscape homogenization due to agricultural practices intensification could affect the fledging success. Finally, Buck and collaborators (2020) investigated the levels of PBCs and OCs in specimens from the island of Tenerife, showing a reduction in eggshell thickness probably due to the presence of p,p'-DDE in the surface of active and abandoned croplands in a 200 m-radius around the nest and with proximity to urban areas. These data demonstrate the importance to evaluate the different aspects of the reproductive success for a more accurate and effective conservation of the species. A very innovative approach would be to link these parameters with the evaluation of biochemical, cellular and tissue responses of each organism to prevent long-term effects on the individual and the general population.

3.6.9. Risk assessment

The risk assessment was carried out in 4 articles (Table 5), and different methods were applied (Toxic equivalency, TEQ; Hazard quotient, HQ; Bioaccumulation factor, BAF; Toxicity-Exposure-Ratio, TER). These studies showed that contaminants such as PCDD/Fs, PCBs, Dioxin-like PCBs, PBDEs, and DDTs represent a potentially high hazard for common kestrel (Yin et al., 2018; L. Yu et al., 2013; Zhang et al., 2021).

Table 4

Studies that met the criteria for inclusion in this review, divided by reproductive success parameters evaluated. The animal's sex was not specified, and all the indicated studies were conducted in the field. The abbreviation ns means "not specified", the symbols "+" and "-" indicate an effect or no effect, respectively. Asterisk (*) refers to indicative results extrapolated from the graphs.

Life stage	Biological material	Contaminant	Human exploited environments	Methodological approach	Sample size	Results (mean \pm sd; min-max)	Unit of Measurement	Reference, year
Eggs	Egg	DDTs, PCB		Eggshell thickness	40	+ 0.14–0.22 *	mm	Buck et al. (2020)
				Fledging success		-		
				Hatching success		-		
Nestlings	Animal		Agricultural	Fledging success		+		Sumasgutner et al. (2019)
ns	Egg		Urban	Clutch size	157 broods	-		Sumasgutner et al. (2014)
				Hatching success		+		
Nestlings	Animal			Number of fledglings		+		
				Fledging success		-		
Eggs	Egg		Agricultural	Clutch size	109	- 5.39 \pm 0.15	n	Costantini et al. (2014)
				Egg volume	341	- 56.62 \pm 0.28	cm ³	
				Hatching success	107	- 81.32 \pm 5.04	%	
Nestlings	Animal			Number of fledglings	113	- 4.25 \pm 0.28	n	
				Fledging success	106	- 96.56 \pm 1.67	%	

Table 5

Studies that met the criteria for inclusion in this review, divided by risk assessment parameters evaluated. The abbreviation ns means “not specified”, the symbols “+” and “-” indicate an effect or no effect, respectively. Asterisk (*) refers to indicative results extrapolated from the graphs.

Life stage	Sex	Study type	Biological material	Contaminant	Methodological approach	Sample size	Results (min-max)	Unit of Measurement	Reference, year		
Juveniles	F	Field	Muscle	PCDD/Fs, PCBs and Dioxin-like PCBs	TEQ	25	+ 0.03–0.6 *	pg/g l.w.	Zhang et al. (2021)		
	M						+ 0.03–0.09 *			ng/g w.w.	
Adults	F	Field	Animal	PBDEs	HQ	17	+ 0.01–0.5 *	ng/g w.w.	Yin et al. (2018)		
	M						+ 0.05–0.8 *			ng/g w.w.	
ns	ns	Field	Liver	DDTs	HQ	23	+ 0.15		Yu et al. (2013)		
ns	ns	Field	Liver	PCBs			- 0.042				
				PBDEs			- 0.067				
Adults	ns	Laboratory	Animal	Brodifacoum	BAF	2	- 0.05		Hromada et al. (2011)		
	ns						Warfarin			BAF	- 0.04
	M						Brodifacoum			TER	- 24.76
	M						Warfarin			TER	- 571.3
	F						Brodifacoum			TER	- 45.61
	F						Warfarin			TER	- 4285.7

On the other hand, Hromada and collaborators (2011) showed a low risk for kestrel linked to secondary exposure to rodenticides.

The results of these works show that further investigations are necessary on the risk assessment, considering not only the presence of contaminants but also the biochemical responses. Indeed, to identify the different risks to which *Falco tinnunculus* may be exposed and, therefore, to obtain useful information for decision-making, a proper risk assessment procedure should consider as much endpoints as possible.

The results of these works show that further investigations are necessary on the risk assessment, considering not only the presence of contaminants but also their effects by biochemical responses. Indeed, to identify the different risks to which *Falco tinnunculus* may be exposed and, therefore, to obtain useful information for decision-making, a proper risk assessment procedure should consider as much endpoints as possible. Moreover, in this procedure, should be considered also multiple stressors, such as contamination, climate change, poisoning, and habitat reduction, that affect not only the individual but also the population of common kestrel. This is important also to predict the combined effects of stress factors on common kestrel.

4. Conclusions

This review underlines some important gaps in the knowledge of the toxicological status of the common kestrel and from the gaps some suggestions for future research and monitoring studies arise. The first gap concerns the fact that papers on the toxicology of this species are in limited number and concentrated in some specific geographical areas, while in some area's information are almost completely lacking. There is then a need for increase the monitoring activity in general and particularly in some areas such as Africa and Asia continents and in some European countries like Italy and Germany.

A second important point to be considered is that non-lethal monitoring approaches should be developed and conducted, through in vitro and in vivo analysis using non or low-invasive sampling and monitoring methods. This approach allows to investigate alive and healthy animals and to have a realistic picture of the toxicological status of wild populations. Not only legislative or ethical reasons support this choice, but also scientific, including the potential for monitoring high numbers of specimens for repeating sampling several times over the time even in the same specimen or population. *In vitro* testing can help unravelling mechanisms that are at the base of the toxicology of the common kestrel and identifying compounds or mixtures to which the species is particularly sensitive. Excellent materials are blood, excreta, and feathers. Monitoring contamination in dead animals can give only a partial information and do not help in the study of sublethal and long-term toxicological effects.

A further gap of knowledge concerns the lack of information on the

presence and effects of emerging contaminants in the common kestrel, suggesting the specific need to implement in vitro and in vivo studies on this subject.

It was also highlighted the lack of knowledge and therefore the need to significantly increase the studies relating to the sublethal effects of contaminants in this species. Studies on different effects such as genotoxicity, neurotoxicity, on the immune system and estrogenicity, should be conducted in a non-lethal way for the species.

But what is configured as the fundamental aspect and the most important gap to be bridged regarding the toxicology of this species and of avian species in general, concerns the development and application of a multitasking approach. It would allow to integrate information at different levels of biological organization, from the molecular and cellular and in general sub individual level, to the individual and population, including data on chemical contamination and on the environment in which the different populations live. As we have seen in this paper, to date the studies have essentially focused on specific aspects of toxicology, while an integrated approach could permit to have a complete picture of the health status of this species in the different environments. Risk assessment should be conducted considering the whole set of information and not just a few endpoints.

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CRedit authorship contribution statement

Silvia Casini: Conceptualization, Supervision, Validation, Writing – review & editing; **Ilaria Caliani:** Conceptualization, Methodology, Writing – original draft, Supervision; **Laura Giovanetti:** Methodology, Investigation, Formal analysis, Data curation, Writing – original draft; **Tommaso Campani:** Methodology, Data curation.

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.

Data Availability

The data that has been used is confidential.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.etap.2023.104237](https://doi.org/10.1016/j.etap.2023.104237).

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