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Effect of Pesticides on Animals in Forest of India

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ABSTRACT: The impacts of pesticides on wildlife are extensive, and expose animals in urban, suburban, and rural areas to unnecessary risks. Beyond Pesticides defines "wildlife" as any organism that is not domesticated or used in a lab. This includes, but is not limited to, bees, birds, small mammals, fish, other aquatic organisms, and the biota within soil. Wildlife can be impacted by pesticides through their direct or indirect application, such as pesticide drift, secondary poisoning, runoff into local water bodies, or groundwater contamination. It is possible that some animals could be sprayed directly; others consume plants or prey that have been exposed to pesticides.

KEYWORDS: pesticides, forest, wildlife, water bodies, groundwater, India, animals, fish, mammals, birds

I.INTRODUCTION

Pesticide exposure can be linked to cancer, endocrine disruption, reproductive effects, neurotoxicity, kidney and liver damage, birth defects, and developmental changes in a wide range of species. Exposure to pesticides can also alter an organism's behavior, impacting its ability to survive. In birds, for example, exposure to certain pesticides can impede singing ability, making it difficult to attract mates and reproduce. Pesticides can also affect birds' ability to care for offspring, causing their young to die. For bees, even "near-infinitesimal" levels of systemic pesticides result in sublethal effects, impacting mobility, feeding behaviors, and navigation. [1,2]

Many deformations have been found after exposure to hormone-mimicking pesticides classified as endocrine disruptors. The impacts of these chemicals include hermaphroditic deformities in frogs, pseudo-hermaphrodite polar bears with penis-like stumps, panthers with atrophied testicles, and intersex fish in rivers throughout India. Reproductive abnormalities have been observed in mammals, birds, reptiles, fish, and mollusks at exposure levels considered "safe". The estimated economic costs of losses to biodiversity[3,4] — for the value of pollinator services, "beneficial" predators, and birds and aquatic life — are continually changing as more complex and comprehensive studies are published. Earlier studies estimated that the cost of losses to biodiversity might amount to more than \$1.1 billion annually. Now, we know that the loss of biodiversity can cost hundreds of billions of dollars annually. Natural pest control, a fundamental agricultural service,[5,6,7] is estimated to be worth \$100 billion annually. The role of soil biota in increasing agricultural productivity is worth \$25 billion annually. By 2009, the value of dependent crops attributed to all insect pollination was estimated to be worth \$15.12 billion annually. Other economic impacts are related to the recreational use of wildlife. Indian citizens already spend over \$60 billion annually on hunting, fishing, and observing [8,9]wildlife; much of the wildlife at the center of those activities depends on insects as a food source. Researchers have found that there is a steady decline in these insects due to pesticide exposure and an overall decline in biodiversity[10,11]. It could be concluded then that, as beneficial insect populations decline, their ability to provide ecosystem services will also decline, impacting the available wildlife for hunting, fishing and observing. The demand for these recreational activities will stay constant while the supply (availability) will decline, causing an increase in dollars spent by Indian citizens for each year. Two ways to combat the negative impacts of pesticides on wildlife are: to implement organic practices for your own lawn and garden, and to support organic agriculture, rather than on conventional agriculture, which relies on pesticide use. [12,13,14]Beyond Pesticides supports organic agriculture as effecting good land stewardship and reducing wildlife's hazardous chemical exposures. The pesticide reform movement, citing pesticide problems associated with chemical agriculture — from groundwater contamination and runoff to drift — views organic as the solution to these serious environmental threats.[15,16]

Conventional agriculture relies on a "pick and choose" method when it comes to pesticide use — only treating the symptoms of bad land management instead of acknowledging the deeper problems and attempting to understand agriculture as a whole system, including impacts on wildlife. Adopting a whole-systems approach, starting with management methods that "feed-the-soil," and thus, promote healthy land from the ground up, would result in the

greatest systemic benefit. Beyond Pesticides has long supported a “feed-the-soil” approach to agricultural management. This systems approach, which centers on managing soil health and on proper fertilization, eliminates synthetic fertilizers and focuses on building the soil food web and nurturing soil microorganisms. Experience demonstrates that this approach develops a soil environment rich in microbiology, which will produce resilient, productive land and benefit wildlife.[17,18]

Healthy, resilient soil reduces any need for pesticides; terrain free from pesticides benefits wildlife and promotes natural predators, who can then do what they were meant to do in nature — provide natural controls. Organic systems save wildlife from the dangerous impacts of pesticides, encourage them to flourish, and restores the natural balance that is unable to exist in a conventional agricultural system. One way that groups like Beyond Pesticides have sought to protect wildlife from the threat of pesticides is by holding federal agencies accountable to the Endangered Species Act (ESA) of 1973, which provides for the conservation of ecosystems on which threatened and endangered species of fish, wildlife, and plants depend. EPA has routinely disregarded the ESA’s requirement to consult with federal wildlife agencies on how to implement conservation measures to protect threatened and endangered species from pesticides. After years of gridlock, federal wildlife agencies, EPA, and the U.S. Department of Agriculture (USDA) asked the National Academy of Sciences to study the issue and report on best ways to protect listed species (any species likely to become endangered or which is in danger of extinction) from the effects of toxic pesticides.[19,20] The National Academy of Sciences report identified deficiencies for all the agencies involved in pesticide consultations, but singled out the EPA’s approach for its numerous analytical shortcomings. In response to the Academy’s recommendations, the agency announced several reforms, in the fall of 2013, designed to protect endangered species more effectively. [21,22,23] Though the ESA is one of the most important laws for protecting wildlife, the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Clean Water Act (CWA), and National Environmental Policy Act (NEPA) are other significant laws meant to keep wildlife safe. FIFRA regulates pesticides to prevent “unreasonable adverse effects” to humans and the environment, including wildlife. The stated objective of the CWA is to “restore and maintain the chemical, physical, and biological integrity of the Nation’s waters [24,25,26]. . . for the protection and propagation of fish, shellfish, and wildlife.” Finally, NEPA requires that any federal government action that may impact wildlife and the environment must review and evaluate those impacts before any action is taken. Each of these laws can be utilized to protect wildlife by holding federal agencies accountable to them. [27,28]

II.DISCUSSION

There are many studies concerning the effects of various pesticides on certain living organisms. All of them show a negative xenobiotics effect.[29,30] Majority of studies was conducted on mice and rats, which are the main model objects for toxicological studies on pesticide effects.

Chlorpyrifos is a broad-spectrum insecticide that interfere with signaling from the neurotransmitter acetylcholine [16,17]. It causes microflora dysbiosis and, consequently, leads to a change in the level of microorganisms metabolites [18,19,20]. Also, it is often found in food, thereby affecting the normal functioning of the endocrine and gastrointestinal systems [21]. It was shown on rats orally fed with special diet with different fat contents [22]. In parallel with the altered nutrition, rats were injected with chlorpyrifos.[31,32] Chronic introduction of the pesticide caused reduction of luteinizing, follicle-stimulating hormones and testosterone concentrations on a diet with a normal fat content. A high-fat diet and the effect of the pesticide have increased the number of anti-inflammatory cytokines. Pesticide-induced abnormalities in the intestinal microbiome were more apparent in rats fed a high-fat diet. The affected bacteria included short-chain fatty acid-producing bacteria, testosterone-related genus, pathogenic bacteria, and inflammation-related bacteria. The study helped clarify the relationship between disrupted endocrine function and gut microbiota dysbiosis induced by pesticides.[33,34,35]

Liang et al. also studied the effect of a similar diet containing chlorpyrifos on mice. Subsequently, antibiotic treatment and microflora transplantation were performed [23]. The results of the experiment showed a violation of the intestinal barrier by chlorpyrifos, which led to increased penetration of lipopolysaccharides into the body and, consequently, the appearance of inflammation in the intestine. In addition, the mice that received the microbiota modified with chlorpyrifos acquired a fat mass and low insulin sensitivity.[36,37,38]

Another study revealed the effect of propamocarb fungicide on the microflora and intestinal metabolism of mice [24]. Analysis of the operational taxonomic unit (OTU) showed that 32.2% of the cecum OTUs was changed after exposure to propamocarb. In a similar experiment, the fungicide imazalil was used [25]. The microbiota of the contents

of the cecum and feces changed at phylum and genus levels after application of the fungicide, this showed the sequencing of 16S rRNA. Operational taxonomic unit (OTU) analysis showed that 14.0% of fecal OTUs and 31.1% of cecal OTUs changed after imazalil exposure. The results showed that high doses of fungicide disrupt the metabolism of mice by altering the intestinal microbiota. In addition, imazalil significantly increased the number of bacterial infections in the mucous membrane of the colon of mice, affecting the intestinal barrier function.[39,40,41]

A decrease in abundance and bacterial diversity was also shown after consumption of systemic triazole fungicide penconazole [26]. In mice treated with penconazole and its enantiomers, the relative content of the microbiota in the intestine, in particular, the cecum, changed. The relative abundances of seven gut microflora (at the genus level) were altered following exposure to penconazole. Penconazole caused significant changes in the relative abundances of five gut microflora. Metabolism analysis showed metabolic profile disturbance after exposure to this substance. This indicates the harmful effects of this pesticide on the animals.[42,43,44]

In addition to studying the effect of pesticides on the intestinal microbiota, studies have been conducted on the combined use of antibiotics and pesticides. The use of antibiotics directly affects the intestinal microbiota, reducing the number and diversity of bacteria [27,28]. In turn, the intestinal microbiota changed by antibiotics can affect the chemical transformation of xenobiotics in the body [29,30]. Finding the relationship between the effects of antibiotics and pesticides have been conducted where rats with an antibiotic-modified microbiota were exposed to triazine herbicides [31]. The results showed that antibiotic administration reduces the number of bacteria in rats: The relative abundance of Ruminococcaceae species decreased, and the Bacteroides species increased. It was also found that antibiotics suppress the gene expression of hepatic metabolic enzymes and increased the expression of proteins associated with intestinal adsorption. All this together increases the risk of exposure to triazine herbicides (atrazine, simazine, ametrine, terbutylazine, and metribuzin) on the body, leading to an increase in their bioavailability.[45,46,47]

In modern studies [32,33,34], the bacterial composition of soils is used as one of the important indicators of the negative consequences of pesticides usage. Pesticides have been shown to act on bacteria that fix nitrogen [35], cause changes in plant probiotic soil microflora [36] and total soil microbial diversity [37]. It is little-known about the effect of fungicides on the bacterial community of the soil. The effect of different concentrations of azoxystrobin fungicide on the soil and intestinal microbiota of soil animals was studied using the example of Enchytraeus crypticus annelid worm [38], as well as the effect of the insecticide monocrotophos on earthworms, which are actively used in studies as a model object for assessing environmental risks from the usage of chemicals [39]. In general, the results are similar: A decrease in the number of beneficial bacteria in the intestine and a decrease in microbiota were shown. Enchytraeus crypticus showed an increase in the spread of proteobacteria in response to an increase in the concentration of azoxystrobin. A significant change in the structure of the soil microbial community was observed under the effect of penconazole, carbendazim, pencycuron, and fludioxonil [40,41]. On adding 10 mg/kg carbendazim, the change in soil microbial composition was greater than 40%. The relative abundance of bacteria also significantly changed under the influence of penconazole, pencycuron and fludioxonil.[48,49,50]

III.RESULTS

Trichlorfon insecticide is often used in agriculture and horticulture [42]. This compound is actively used to combat various parasitic infections in aquaculture. It is well soluble in water, and, accordingly, the excessive use of such a phosphorus-containing pesticide leads to environmental pollution [43]. The effect of various concentrations of trichlorfon was identified on the intestinal microbiome of common carp Cyprinus carpio [44]. Exposure to the pesticide significantly reduced the height of intestinal villi, and also reduced the level of gene expression for claudin-2, occludin, ZO-1.[51,52,53] Exposure to trichlorfon influenced the composition of the microbiota community and reduced the diversity of bacteria in the intestines of carp. The proportions of probiotic bacteria, namely, Bifidobacterium, Akkermansia, and Lactobacillus, were observed to be reduced after trichlorfon exposure. Together, this proves the negative effect of trichlorfon: It can damage the intestinal barrier, cause oxidative damage to the intestine, cause an inflammatory reaction and change the structure of the intestinal microbiota in carp.[54,55,56]

The massive usage of pesticides leads to a change in fresh algal biocenoses, causing water blooming. Azoxystrobin is a widely used broad-spectrum strobilurin fungicide. It has been shown that azoxystrobin stimulates the growth of cyanobacteria by inhibiting the growth of competitive organisms, for example, Chlorophyta, and also inhibits the growth of parasitic cyanobacteria, fungi, pathogenic bacteria, and viruses [45].

One of the most popular herbicides used worldwide for weed control is glyphosate [52,53,54]. It is actively used on islands near the coastline, that effects on surrounding marine and coastal species [55,56]. Various studies have been conducted to study the effect of this pesticide on the intestinal microflora of animals. For example, in Hawaiian green turtles, under different concentrations of the pesticide, a decrease in density and inhibition of bacterial growth were observed [57]. This fact indicates the adverse effect of glyphosate on the general condition of the animal. In other species (Chinese mitten crab), [57,58,59] the antioxidant ability of the intestine decreased with the effect of the pesticide and the content of malondialdehyde increased. As a result of sequencing, an analysis was performed that showed that glyphosate reduced the diversity of the intestinal microbiota of the Chinese mitten crab, and the taxonomic richness of bacteroids and proteobacteria increased significantly [58]. Glyphosate, absorbed with food, directly contacted with the intestinal microbiota of pollinating insects. Quantitative PCR revealed a significant change in the composition of the intestinal microflora [59], the appearance of an unbalanced microbiota, which reduces the bee's resistance to pathogens. Glyphosate caused a strong decrease in the bacteria *Snodgrassella alvi*, a partial decrease in *Gilliamella apicola* and an increase in *Lactobacillus* spp.[60,61,62]

In addition to the glyphosate effect on the growth of intestinal bacteria, this pesticide has an effect on the synthesis of amino acids (shikimate pathway), in particular, it inhibits the enzyme 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS). The gene encoding the shikimate pathway enzyme is present in all sequenced genomes of intestinal bacteria of the honeybee [60]. This fact indicates a high potential sensitivity of bacteria (and, consequently, bees) to glyphosate. A decrease in the dominant intestinal microflora of insects effected by glyphosate has been demonstrated. Moreover, the effect of this herbicide increased the mortality rate of bees due to increased susceptibility to the influence of the pathogen *Serratia marcescens*. Thus, glyphosate perturbs the beneficial intestinal microflora of honey bees, potentially affecting their health and pollination efficiency.[63,64,65]

Studies have shown that glyphosate affects both adult insects and larvae. The experimental data showed a decrease in survival rate, development rate, mass of larvae and bacterial diversity of the middle intestine *Apis mellifera* [61]. Xenobiotics in high concentrations have the most negative effect on colonies of immature bees, while adults do not experience stress due to its effect.[66,67,68]

Interrelationship between pesticide and intestinal bacteria that synthesize vitamins was shown. Human MAIT cells (invariant T-cells associated with the mucosa) can respond to vitamin metabolites: Riboflavin and folate. *Escherichia coli* cells were shown to activate MAIT cells, while *Bifidobacterium adolescentis* and *Lactobacillus reuteri* inhibited MAIT cell activation [62]. Exposure to chlorpyrifos significantly increased *E. coli* colonies mediated by the activation of MAIT cells, and the effect of chlorpyrifos together with glyphosate inhibited colonies growth. In this case, proteomic analysis showed that glyphosate had an effect on the biosynthesis of riboflavin and folate. Thus, chlorpyrifos and glyphosate increase the anti-inflammatory immune response.[69,70,71]

A long-term effect of the Roundup herbicide (made from glyphosate) on the microbiota of rat intestines demonstrated the growth of Bacteroidetes bacteria and a decrease in the number of Lactobacillaceae [63]. The culture method showed that Roundup has a direct effect on the intestinal microbiota: Bacteria showed different sensitivity to the herbicide, including the identification of a resistant strain of *Escherichia coli*, which is associated with the absence of the EPSPS gene. Thus, Roundup accumulations in the environment have a significant negative effect on rat health.[72,73,74] Intestinal microbial communities play a crucial role in maintaining the health of animals. We have shown that pesticides can affect the microbiome of animals of various taxonomic groups. It is noteworthy that pesticides of various classes (insecticides, fungicides and herbicides) can affect the intestinal microbiota of animals. Glyphosate has a significant negative effect on the intestinal microbiota, both mammals and pollinators. Changes in the microbiome induced by pesticides ultimately affect the immunity of animals, reproductive ability and even their behavioral characteristics. Pesticides have a significant effect on the taxonomic composition and ratio of bacteria in the gut of bumblebees and bees. Solutions for the correction of pollinator microbiome are needed.[75,76,77]

IV.CONCLUSIONS

In recent decades an increase in the use of pesticides to protect plants from pests, diseases and weeds has been observed. There are many studies on the effects of various pesticides on non-target organisms. We should analyze and summarize published scientific data on the effects of pesticides on the animal microbiome. [78,79,80] Pesticides can affect various parameters of the animal microbiome, such as the taxonomic composition of bacteria, bacterial biodiversity, and bacterial ratios and modify the microbiome of various organisms from insects to mammals. Pesticide induced changes in the microbiome reducing the animal's immunity. The negative effects of pesticides could pose a global problem for

pollinators. Another possible negative effect of pesticides is the impact of pesticides on the intestinal microbiota of bumblebees and bees that increase the body's sensitivity to pathogenic microflora, which leads to the death of insects. In addition, pesticides can affect vitality, mating success and characteristics of offspring.[81,82]

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