Technical Report No: PR - 249

National centre for surveillance and monitoring of impact of environmental contaminants on ecosystem components with special focus on birds in India

Principal Investigator
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SACON

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September 2023

Project Title: National centre for surveillance and monitoring of impact of environmental contaminants on ecosystem components with special focus on birds in India.

Funding Agency: Ministry of Environment, Forest and Climate Change, Govt. of India.

Published by: Sálim Ali Centre for Ornithology and Natural History, South India centre of Wildlife Institute of India, (Ministry of Environment, Forest and Climate Change, Government of India) Anaikatty, Coimbatore 641 108, Tamil Nadu.

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Suggested citation: Muralidharan S, Nambirajan K, Kirubhanandhini V, Bhagyasree V, Neenu Purusothaman, Gayathri V & Kaja Maideen A (2023). Technical report, National centre for surveillance and monitoring of impact of environmental contaminants on ecosystem components with special focus on birds in India. Sálim Ali Centre for Ornithology and Natural History, South India Centre of Wildlife Institute of India, Technical report. SACON PR - 249 Pp- 175.

Project Duration: October 2017 to March 2021

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Submitted to
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Acknowledgements

We are grateful to the Ministry of Environment, Forest and Climate Change (MoEF&CC), Government of India for extending financial support to this project. We thank all officials at the MoEFCC, particularly Dr. T Chandini, Advisor (Retd.), for their cooperation towards execution of the project.

We are thankful to the Principal Chief Conservators of Forest and Chief Wildlife Wardens of Andhra Pradesh, Odisha, Tamil Nadu, Gujarat, Rajasthan, Uttar Pradesh, Bihar, West Bengal, Telangana, Karnataka, Maharashtra and Puducherry for granting permits to collect samples of dead birds for the laboratory investigations. We are beholden to also the concerned District Forest Officers in the referred states for their cooperation.

We are grateful to Shri. Virendra Tiwari, IFS, Director, Sálim Ali Centre for Ornithology and Natural History, South India Centre of Wildlife Institute of India, Coimbatore for his support.

We are thankful to Dr. K Sankar, former Director, SACON for his cooperation and advice throughout the project period. We thank all faculty members, research scholars, librarian, finance and administrative officials of SACON for their cooperation.

Mr. K Chandrasekar, Senior Scientist, Agilent Technologies, gave us necessary inputs in setting up analytical facility at the division, and we are grateful to him.

We are indebted to Dr. K Bhuvaneshwari, Professor, Pesticide Residue Laboratory, and Dr. S Avudainayagam, Professor (Retd), Department of Environmental Science, Tamil Nadu Agriculture University, Coimbatore for their technical support.

We thank Ms. Gira Shah, Managing Trustee, Mr. Kartik Shastri, Trustee, Dr. Shashikant Jadhav, and, Mr. Sumeet Gurbani, Jivdaya Charitable Trust, Ahmedabad for rendering help in sample collection, especially during the kite flying festival in Ahmedabad, Gujarat.

We thank Dr. K Ashokan, Veterinary Surgeon, Sathyamangalam Tiger Reserve and Mr. S Bharathidasan, Secretary, Arulagam, Coimbatore for helping us in getting samples of dead birds for toxicological investigations.

We express our heartfelt gratitude to the Chairman and Members of the Research, Monitoring and Advisory Committee (RMAC) of SACON for their critical review at crucial stages which helped us to make mid-course corrections. Dr. P R Arun, Research Coordinator needs a mention for his support.

We acknowledge the support extended by all the former students of Ecotoxicology Division, SACON, particularly Drs. R Jayakumar, S Jayakumar, V Dhananjayan, P Jayanthi and Mythreyi Devarajan.

We very much appreciate the help of other research students of Ecotoxicology division, namely Messrs. Sharma K, L Kalaiarasu, Gourav Sonawane, Gaurav S Singh, Tejas Karmarkar, Gautam S. Kadam, Aditya Roy, Misses. E Jeevitha, R Madhumita and Rithika for their help in the field and laboratory. We also thank Mr. S. Vigneshwaran for his help in formatting and designing the report.

We thank laboratory interns Messrs. S Gokul, K Vijayganesh, B Abhishek, V Revanth and D Tamilvanan for their help in the laboratory.

Mr. T Manikandan, Laboratory Assistant, SACON lent untiring support in collecting samples in the field and processing large number of samples in the laboratory.

We acknowledge everyone who provided information on mortality of birds in different parts of India, and also sent samples of dead birds for investigation.

If we have missed out anyone to thank by name, we offer our apologies.

Terms and Abbreviations used in the Report

ANOVA	Analysis of Variance
BDL	Below Detection Limit
CB	Carbamate
CIBRC	Central Insecticide Board and Registration Committee
DDT	Dichlorodiphenyltrichlororthane
DPPQS	Directorate of Plant Protection, Quarantine & Storage
EC	European Commission
EU	European Union
F	Fungicide
FAO	Food and Agriculture Organization of the United Nations
FSSAI	Food Safety and Standards Authority of India
G	Granule
g	Gram
GC-MS/MS	Gas Chromatography –Mass Spectrometry/Mass Spectrometry
H	Herbicide
ha	Hectares
HCH	Hexachlorocyclohexane
I	Insecticide
IBA	Important Bird Area
IUCN	International Union for Conservation of Nature
Kg/ha	Kilo gram per hectare
LC50	Lethal Concentration
LC-MS/MS	Liquid Chromatography-Mass Spectrometry/Mass Spectrometry
LD50	Lethal dose
LOD	Limit of Detection
LOQ	Limit of Quantification
m	Meter
mg	Milligram
mg/kg	Milligram per kilogram
MRM	Multiple Reaction Monitoring
MT	Metric Ton
NCAE	National Centre for Avian Ecotoxicology
ng/g	Nanogram per gram
OC	Organochlorine
OCP	Organochlorine Pesticide
OP	Organophosphate
p,p'-DDD	Dichlorodiphenyldichloroethane
p,p'-DDE	Dichlorodiphennyldichloroethylene
p,p'-DDT	Dichlorodiphenyltrichloroethane
POPs	Persistent Organic pollutants

Ppb	Parts per billion
Ppm	Parts per million
PPPs	Plant Production Products
RPM	Rotation per minute
SACON	Sálim Ali Centre for Ornithology and Natural History
SD	Standard deviation
SE	Standard error
Sq.km	Square kilometre
TCP	Total Chlorinated Pesticides
USEPA	United states environmental agency
WHO	World Health Organisation
α-HCH	Alpha-Hexachlorocyclohexane
β-HCH	Beta-Hexachlorocyclohexane
γ-HCH	Gamma-Hexachlorocyclohexane or lindane
δ-HCH	Delta-Hexachlorocyclohexane
λ	Lambda
Σ	Summation
µg/g	Microgram per gram
µg/kg	Microgram per kilogram

CONTENTS

Executive Summary

Ornithologists, the world over accept that population of many species of birds have declined, and that environmental contamination is one of the potential reasons for the same. Birds are widely distributed class of vertebrates, and highly susceptible to vast spectrum of threats, such as climate change, deterioration of habitat quality, habitat loss, invasive species, numerous types of contaminants such as harmful pesticides and heavy metals. In India, population of several species of birds plummeted significantly in recent times. Death of birds due to pesticide poisoning has become rampant of late across agricultural landscapes. What we get to understand is a minuscule percentage of the reality. Many instances go unrecorded unless they attracted the attention of media and public. Studies were conducted at SACON between 2000 and 2016 to document uncommon mortalities of birds across the country, and recognise the chemicals responsible. During the referred period, SACON received hundreds of dead birds comprising 130 species. Although, incidences of poisoning were confirmed in 17 species of birds, on many occasions we could not identify any chemical responsible for poisoning and conduct inclusive studies due to lack of in-house analytical facility. In this context, the current project was initiated with the following objectives; 1. Set up a state-of-the-art analytical facility at SACON, 2. Monitor residue levels of problem chemicals in select species of birds in India, and generate a database, 3. Identify chemicals responsible for mortality of birds across the country and 4. Assess the effectiveness of acts, guidelines on usage of chemicals in the country. While the entire country was the study area, Andhra Pradesh and Odisha were considered as intensive study areas under phase I. The project output and research findings are presented and discussed under separate chapters.

Establishment of National Centre for Avian Ecotoxicology

Analytical facility with bare minimum pieces of equipment existed at SACON since 1993 to study ill effects of pesticides, heavy metals, PCBs, PAHs and pharmaceutical drugs on birds. Invention of newer chemical molecules, and their entry into the environment have been an ongoing progression, and we faced serious constraints to assess the impact of contemporary chemicals on the environment. This was largely because the analytical facility in the division of Ecotoxicology had become obsolete and irrelevant to the present context. Studies related to environmental contamination need a technically and technologically sound analytical instrumentation facility. Incidentally to cater to the changing demands of national and international statutory agencies, every facility would need up-gradation periodically. Under this circumstance, it became imperative to augment the existing facility at SACON with state-of-the-art instruments. Hence, a modern analytical facility was set up with advanced pieces of equipment, viz, i) Gas Chromatograph - Mass Spectrometer (GC-

MSMS), ii) Gas Chromatograph - Electron Capture Detector (GC-ECD), iii) Liquid Chromatograph - Mass Spectrometer (LC-MSMS) and iv) Inductively Coupled - Plasma Mass Spectrometer (ICP-MS). In addition to the above-referred major analytical instruments, a few minor instruments and necessary paraphernalia were also purchased and fixed to make the laboratory fully functional with state-of-art gadgets. The facility named as "**National Centre for Avian Ecotoxicology**" was inaugurated by Shri Prakash Javadekar, the then Honourable Minister for EF&CC, Government of India on the 24th August 2019. Subsequently, all the equipment were calibrated, extraction and digestion procedures optimized to qualify and quantify numerous contaminants in diverse environmental matrices.

Impact of pesticides on birds in India

Efforts were made to collect and receive samples of dead birds from all over India. Opportunistic sampling strategy and organized field visits were adopted for the same. Efforts were also made to trap a few birds in select locations. Between January 2018 and March 2021, totally 700 birds comprising 95 species were collected dead from 12 states/union territory of India, namely Andhra Pradesh, Odisha, Tamil Nadu, Gujarat, Rajasthan, Uttar Pradesh, Bihar, West Bengal, Telangana, Karnataka, Maharashtra and Puducherry during the project period for toxicological investigations. Postmortem examinations were conducted either in the field or at SACON laboratory. Tissues such as muscle, liver, kidney, gut content and suspected baits were collected, and brought to SACON over ice, and preserved at -20°C until chemical analyses were performed. Multi-residue extraction procedure was adopted to extract residues of pesticides and drugs. Samples for heavy metal analysis were prepared using Microwave Digestion System. While pesticides and drugs were analysed using GC, GC-MSMS and LC-MSMS, heavy metals were analysed using ICP-MS Residue levels are expressed as ng/g ±SE (wet weight). Findings in this chapter are presented under three sub-headings, i) Confirmed poisoning incidents in birds, ii) Mass mortality of birds due to reason other than poisoning, and iii) Background concentrations of pesticide residues in birds.

i) Confirmed poisoning incidents in birds: During the project period, 26 suspected poisoning cases involving 26 species of birds were investigated. While poisoning among birds have been dealt separately, data on pesticide residues have been compiled to check the overall load, variation among species, tissues and feeding habits to understand the implications with reference to usage patterns of pesticides, drugs and policies of the government that are in existence. While 24 incidents were confirmed to be pesticide poisoning, remaining two were Nimesulide poisoning. Species of birds succumbed include, Sarus Crane, White-rumped Vulture, Indian Peafowl, Black Kite, Rock Dove, Comb Duck, Common Myna, Asian Koel, Coppersmith Barbet, Cotton Teal, Demoiselle Crane, Eurasian Sky-

lark, Greylag Goose, Green Bee-eater, Grey-headed Swamp Hen, House Crow, Northern Pintail, Red-vented Bulbul, Yellow-billed Babbler, Indian Pond Heron, Common Moorhen, Changeable Hawk-eagle, Alexandrine Parakeet, Red-wattled Lapwing, Laughing Dove and Cattle Egret. Among these species Sarus Crane falls under the IUCN Vulnerable category and the White-rumped Vulture under Critically Endangered category. Among the species, Peafowl was the most affected species due to poisoning followed by Rock Dove. The Peafowl in India faces severe threat due to poisoning by the farmers in the process of protecting crops.

The poisoning incidents happened in 21 locations in seven states and a union territory (Puducherry). It will be incorrect to presume that the birds in other states are safe. It is just that either our focus was inadequate or media and local people did not take cognisance of the problem. Totally six pesticides, namely Monocrotophos, Carbofuran, Phorate, Chlorpyrifos, Triazophos and Dichlorvos were involved in the poisoning. Majority of the poisoning incidents were due to Monocrotophos followed by Carbofuran. Fortunately, among the six pesticides, three, namely Phorate, Triazophos and Dichlorvos were banned in 2020 by the Indian Government. The other three pesticides, namely Monocrotophos, Carbofuran and Chlorpyrifos are continued to be used in the country, and it is a cause for serious concern. It is to be noted that Monocrotophos and Carbofuran are highly toxic, and are banned in most of the developed countries. These pesticides should be banned or restricted for use in India too to reduce harm to birds.

ii) Mass mortality of birds due to reason other than poisoning: Between 2018 and March 2021, seven episodes of mass mortality of birds involving different species of birds in five states of India, namely Rajasthan, Gujarat, Tamil Nadu, Karnataka and Bihar were investigated. Initial approach was to check if there was any poisoning involved. Subsequently, other possible causes were checked. Notable incidents investigated are; Mortality of water birds in Sambhar Lake, Rajasthan (Botulism), Colonial water birds in Koonthankulam Bird Sanctuary, Tamil Nadu (storm), Spot-billed Pelican in Kokrebellur, Karnataka (Contracaecum sp - infestation), and Eurasian Collared-dove in Jodhpur, Rajasthan (unknown).

iii) Residues of pesticides in the tissues of dead birds collected from different states in India between January 2018 and March 2021: Totally 433 birds consisting 74 species were considered for background pesticide residue analysis. Among the birds analysed, 80.6% of birds belonging to 69 species of birds, had residues of one or more pesticides in their tissues. Five species of birds, namely Indian Cormorant, Laughing Dove, Ruddy Shelduck, Yellow-footed Green-pigeon and Northern Shoveler did not have residues of any of the pesticides studied. Individual pesticide levels recorded ranged between BDL and 13,804 ng/g (DDE in liver tissues of Barn Owl). Similarly, total pesticide load recorded ranged

between BDL in many birds and 14,149 ng/g in liver tissue of the same Barn Owl.

Among the 67 pesticides analysed, 21 pesticides, namely HCH, DDT, Dieldrin, Dicofol, Heptachlor, Endosulfan, Endrin, Chlorpyrifos, Quinalphos, Fenthion, Phenthoate, Profenofos, Methyl Parathion, Cyhalothrin, Permethrin, Cypermethrin, Bifenthrin, Fenvalerate, Thiamethoxam, Butachlor and Metalaxyl, were detected in one or more tissues of the birds studied. DDT (68.6%) was the most frequently detected pesticide, followed by HCH (33.9%) and Chlorpyrifos (19.9%). Seven pesticides, namely Fenthion, Methyl Parathion, Bifenthrin, Fenvalerate, Thiamethoxam, Butachlor and Metalaxyl were the least detected (0.02%). Percentage of detection significantly varied among pesticides. DDT contributed the most to the total pesticide load followed by HCH. High frequency of detection of DDT and HCH may be due to their extensive use in the past and their high persistence.

The variation in concentration among the pesticides was statistically significant ($p<0.05$). Accumulation pattern was in the order of DDT > HCH > Chlorpyrifos > Dieldrin. High levels of DDT and HCH recorded in the study among the pesticides analysed is similar to the previous studies in different species of birds in India and elsewhere. Accumulation pattern of total pesticide load among the organs was in the order of liver > gut content > kidney > muscle. Among the birds studied, while Barn Owl had the highest load of pesticide residues followed by Spotted Owlet, Rose-ringed Parakeet had the least load of pesticide residues in their tissues followed by Eurasian Collared-dove. Barn Owl, Great White Pelican, and Spotted Owlet had more than 800 ng/g of total pesticide load. House Crow, Northern Pintail, Black Kite, Red-naped Ibis and Asian Open-bill had total pesticide load more than 200 ng/g. Thirteen species of birds had more than 100 ng/g of total pesticide load in their tissues. There were no pesticide residues detected in a few species of birds such as Common Babbler, Common Sandpiper, Eurasian Sparrowhawk, Indian Spot-billed Duck, Laughing Dove, Ruddy Shelduck, Yellow-footed Green-pigeon and Northern Shoveler. This may be because of small sample size. Accumulation pattern of total pesticide load among the feeding guilds in the study was in the order of carnivores > omnivores > piscivores > granivores > insectivores.

Residues of pesticides in the tissues of birds collected from Andhra Pradesh: Tissues of 102 dead birds belonging to 23 species of birds were collected for the study from Andhra Pradesh. Out of them, tissues of 87 dead birds comprising 22 species were analysed for pesticides. Among the birds analysed, two species namely, Red-vented Bulbul and Red-wattled Lapwing had none of the pesticides analysed in their tissues. Seventy dead birds belonging to 20 species had one or more pesticide residues in their tissues. Three incidents of pesticide poisoning from Vishakhapatnam, involving three species of birds, namely Cotton Pygmy-goose (carbofuran), Rock Dove and Alexandrine Parakeet (Carbofuran), were attended to. Among the 67 pesticides analysed, residues of ten pesticides namely, HCH,

DDT, Chlorpyrifos, Dicofol, Cypermethrin, Cyhalothrin, Thiamethoxam, Metalaxyl, Carbofuran and Monocrotophos were detected in the tissues of birds analysed. While DDT was the most frequently detected pesticide, three pesticides namely, Dicofol, Thiamethoxam and Metalaxyl were the least detected pesticides. Levels of DDT (110.51 ng/g) was the highest and HCH (28.26 ng/g) the lowest. High prevalence of residues of DDT and its metabolites in the environment in Andhra Pradesh is a point to note.

Residues of pesticides in the tissues of dead birds collected from Odisha: Thirty six dead birds belonging to 15 species collected from Odisha were analysed for pesticide residues. Poisoning incidents involving mortality of two species namely, Black Kite and Northern Pintail from Odisha was also attended and confirmed to be pesticide (Carbofuran) poisoning. None of the pesticides analysed was detected in the tissues of two species, namely Asian Koel and Indian Cormorant. Thirty two birds out of 36 birds analysed had one or more pesticide residues in their tissues. The total pesticide load recorded ranged between BDL in few birds to 2444.8 ng/g in gut content of an Indian Pond Heron. Among the 67 pesticides tested, nine pesticides, namely HCH, DDT, Chlorpyrifos, Quinalphos, Butachlor, Phenthoate, Profenofos, Fenthion and Carbofuran were detected in the tissues of the birds studied. Similar to Andhra Pradesh, DDT (63.9%), HCH (22.2%) and Chlorpyrifos (50%) were the more frequently detected pesticides in Odisha, and it shows their predominant presence in the environment. In general, while the legendary pesticides, namely HCH, DDT, Dieldrin, Endosulfan and Chlorpyrifos had their presence in bird tissues, new generation pesticides were detected largely in crop or gut contents of the birds.

This is the first study that recorded many pesticides in tissues of birds in India. Previous studies had reported only legendary pesticides in birds from India and elsewhere. Hence, there are not many reported values to compare the recorded values of most of the pesticides except legendary organochlorines in the current study. Although, there is ban on many persistent pesticides such as HCH, endosulfan and dieldrin, considering their presence in the environment and restricted use of DDT, regular monitoring of these pesticides along with new generation pesticides is recommended towards conserving birds in India.

Another dimension to the issue is, in India there are not required number of scientists and institutions to work on this complex subject. Documentation of effect of contaminants on birds requires carefully designed field studies and laboratory experiments, which are expensive and time consuming. Classic example is, we took around six years to understand that Diclofenac, a non-steroidal anti-inflammatory drug (NSAID) was responsible for population decline in vultures. Further, ill effects need not be always lethal. Nonlethal effects such as behavioral changes, increased vulnerability to predation, decreased food availability also have to be extensively studied as they can exert long-term impact. Mode of action of new generation chemicals is much different from that of the legendary chemicals.

Hence, we need to be all the more planned and prepared to handle emerging conservation issues in the country.

Investigations on the mortality of White-rumped Vulture

Catastrophic population decline in the White-rumped Vulture *Gyps bengalensis* in India was reported as early as 1990. While diclofenac was reported to have caused the decline, the population of White-rumped Vulture has not recovered even after the drug was banned in 2006 for veterinary use in India. The species is still "at high risk of global extinction", listed as Critically Endangered since 2000, and categorized under Schedule I of Indian Wildlife Protection Act 1972; 2002 (Amended).

Between 2018 and 2019, totally seven White-rumped Vultures were received dead at SACON from four different incidents for toxicological investigation (Nagpur, Sathyamangalam Tiger Reserve, Tamil Nadu, Sanand, and Wild Ass Sanctuary, Dhrangadhra, Gujarat). All were suspected to be poisoning cases based on circumstantial evidences gathered. Since, veterinary use of diclofenac, was the established reason for the mass mortalities of vulture, tissues of kidney, liver, intestine and gut contents of the seven vultures were screened for 13 non-steroidal anti-inflammatory drugs (NSAIDs), namely Diclofenac, Aceclofenac, Ketoprofen, Ibuprofen, Naproxen, Mefenamic Acid, Meloxicam, Nimesulide, Piroxicam, Tolfenamic Acid, Indomethacin, Flunixin, Carprofen and Paracetamol.

Of all the drugs tested, only nimesulide was detected in all the tissues (17 - 1395 ng/g) of vultures collected from Sanand and Wild Ass Sanctuary, Dhrangadhra, Gujarat indicative of exposure. Visceral gout was also observed in all the four vultures during postmortem. Residues of nimesulide in tissues with symptoms of gout, indicated that the vultures died due to nimesulide poisoning. Although, other than diclofenac, many NSAIDs are suspected to be toxic to White-rumped Vultures, only nimesulide is reported in the recent past with clear symptom of gout in wild dead White-rumped Vultures similar to diclofenac. Since, nimesulide appears to act similar to diclofenac in exerting toxic effects, if veterinary use of nimesulide continues, White-rumped Vulture is bound to suffer. It is to be noted that the Ministry of Health and Family Welfare, Govt. of India, based on the recommendations of the Drugs Technical Advisory Board of India through a gazette notification dated 31st July 2023 has banned two toxic veterinary formulations, namely Aceclofenac and Ketoprofen for veterinary use. While this is a welcome news, we hope the Drugs Technical Advisory Board will soon recommend banning Nimesulide too as it has been proved to be toxic to Vultures. Further, an effective system of surveillance and monitoring is recommended to be put in place to collect tissues of vultures and other species of birds for toxicological investigations, and their eventual conservation.

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