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Natural and anthropogenic environmental oestrogens: the scientific basis for risk assessment*

Observations of endocrine effects in wildlife with evidence of their causation

G. Van Der Kraak

Department of Zoology, University of Guelph, Guelph, Ontario, N1G 2W1

Abstract Defining the extent to which environmental chemicals affect the functioning of the endocrine system and thereby contribute to adverse health effects in wildlife is a complex issue. This article provides an overview of case studies in invertebrates and vertebrates demonstrating alterations in aspects of growth, reproduction and development which have been attributed to exposure to endocrine disrupting chemicals (EDCs). The diversity of responses seen with these studies highlights that there will be no single approach applicable to investigating endocrine disruption in wildlife and that understanding the cause and consequence of responses in wildlife is complicated. There is uncertainty in a) making the link between measures of exposure to suspected EDCs and physiological effects, b) interpreting biochemical and physiological effects in relation to whole organism fitness, and c) relating the relevance of responses in the individual to ecologically significant measures such as population sustainability. There will be no short cuts when it comes to defining the risks posed by EDCs. This will be achieved by developing an increased understanding of the biology and physiology of wildlife sentinel species and utilizing epidemiological methodology applied to field monitoring and increased reliance on *in vivo* testing strategies.

INTRODUCTION

Recently there has been great interest in both the scientific community and the general public with respect to the possibility that wildlife have been affected by exposure to chemicals which alter the functioning of the endocrine system. The concept that environmental chemicals influence aspects of growth, reproduction and development in wildlife is not new but has gained renewed attention since the publication of the results of a work session in 1991 entitled 'Chemically Induced Alterations in Sexual Development: the Wildlife/Human Connection' and the book 'Our Stolen Future' (1–2). Studies summarised in these and other workshops have highlighted numerous examples of compromised growth and reproduction, altered development and abnormal behaviour in various taxa of wildlife including invertebrates, fish, amphibians, reptiles, birds and mammals which can be correlated or in some cases causally linked with exposure to endocrine disrupting chemicals (EDCs).

Defining the extent to which environmental chemicals affect the functioning of the endocrine system and thereby contribute to adverse health effects in wildlife has developed into a controversial and highly charged issue. There has been an ever increasing number of studies showing that wildlife have been exposed to chemicals that have the potential to influence the endocrine system and in many cases this is accompanied by physiological changes. How do we interpret this information? How do we establish that these responses are due to EDCs as opposed to other stressors or if the changes are of sufficient magnitude that we should be concerned? The controversy in wildlife studies also stems from the lack of defined criteria for establishing the cause and effects relationships between EDC exposure and

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physiological alterations (3–5). There is uncertainty in a) making the link between measures of exposure to suspected EDCs and physiological effects, b) interpreting biochemical and physiological effects in relation to whole organism fitness, and c) relating the relevance of responses in the individual to ecologically significant measures such as population sustainability. The latter point is significant as from an ecological risk perspective, population level impacts take precedence over individual level effects (6). This represents a major difference in assessing the risk of EDCs in humans versus wildlife.

This chapter will examine the evidence that wildlife populations have been impacted by exposure to EDCs. The first section deals with the underlying complexity and uncertainty related to understanding and evaluating the effects of endocrine disruption in wildlife. The second section summarizes a number of case studies which provide evidence that wildlife populations have been affected by exposure to EDCs. The final section deals with the research needs that must be addressed if we are to improve our understanding of the risks posed by EDCs.

THE SCOPE OF THE PROBLEM

One only has to consider the nature of the endocrine system to begin to understand the complexity of the environmental EDC issue. The endocrine system is involved with chemical communication and serves to regulate and coordinate diverse physiological processes including reproduction, growth, maintenance of the internal environment and energy availability (7). Hormones serve as the chemical messengers and, as is the case for their effects on reproduction, play both an organizational role defining the direction and functional extent of development and an activational role in stimulating changes in the various components of the system. Therefore there is a broad range of effects that may be mediated by environmental chemicals that influence the endocrine system. This may encompass effects which influence the functional integrity of the exposed adult as well as having an impact on development of the offspring. Understanding the potential actions of EDCs in wildlife is further complicated by our limited understanding of the basic endocrinology and physiology of all but a few of these species. Scientists are also dealing with species which use a diversity of reproductive strategies (e.g. oviparity, ovoviparity, viviparity, delayed implantation) and often have unique developmental characteristics (e.g. smoltification, metamorphosis) which generally have unknown sensitivities to EDCs.

Defining the association between chemical exposure and physiological dysfunction most often involves some degree of reasoned speculation. The presence of a compound with an identified mechanism of action in the environment or in a target tissue offers the possibility that such exposure is responsible for the observed adverse effects. This connection is strengthened when the observed effects are consistent with what is known of the mechanism of action of the chemical and when levels of exposure are relevant to these effects. However in the absence of cause and effect information, there is the risk of public and political pressure to eliminate suspected EDCs from the environment when they in fact may have little or no effect on physiological performance.

There has been an increased reliance on the use of physiological endpoints to evaluate the effects of suspected EDCs in wildlife. Cellular and molecular measures such as receptor binding and gene activation tests have been instrumental as screening methods for suspected EDCs and in terms of defining their mechanism of action. Similarly, physiological endpoints such as the measurement of plasma vitellogenin and sex steroid hormone levels are now commonplace in non-mammalian species. However caution is warranted when interpreting these endpoints owing to the uncertainty of whether the biochemical and physiological responses are related to changes in whole organism performance in terms of effects on growth, reproduction or survival. For example, even though endocrine signalling is necessary for proper reproductive functioning, the identification of a hormonal alteration does not necessarily equate with an impairment of reproductive success (8). The impact of such a change on an organism will depend on a number of factors including the hormone affected, degree of impairment, timing of the change and the relative species sensitivity.

An overriding concern with all ecotoxicological investigations is that a variety of factors in addition to endocrine disruption can adversely impact growth, reproduction and survival (Fig. 1). Quite often these are ignored when assessing wildlife populations for the effects of suspected EDCs. Food availability,

disease state, competition and loss of habitat are significant stressors to wildlife, yet our understanding of how these contribute to physiological fitness is often inadequate. Often these factors can impinge directly on many of the endocrine measures and physiological endpoints which are used to evaluate wildlife for the effects of EDCs. A range of other factors including sex, age, season and reproductive state as well as genetics can contribute to variability in these markers. The sampling method and handling and capture stress can also affect physiological measurements and these need to be closely monitored and standardized when sampling wildlife. Interpretation is often complicated in the absence of data on normal ranges for physiological or developmental parameters. Another feature of wildlife studies is that comparisons are often made in relation to preselected reference locations. Given the range of non-chemical factors which influence physiological endpoints there can be questions of whether reference populations are appropriate for comparing responses in exposed populations. Admittedly, this is a concern not only for studies of EDCs but for environmental toxicology in general. Wildlife are subjected to multiple stressors and separating out the incremental risks associated with EDCs relative to other anthropogenic stressors is a major undertaking. In the end, establishing cause and effect relationships responsible for population outcomes and exposure to suspected EDCs or other environmental stressors is essential. With limited resources available for remediation, any errors can have major consequences as remediation efforts can often run into the millions of dollars.

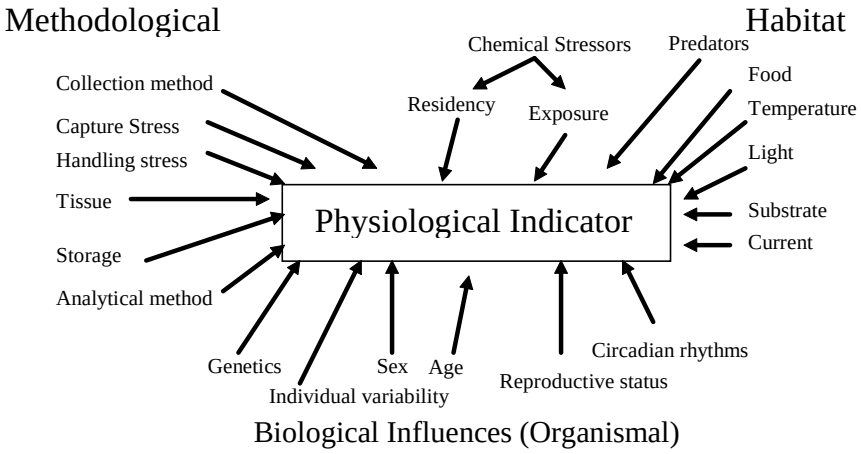

Fig. 1. A summary of various factors influencing physiological endpoints in wildlife.

Several investigators have proposed criteria which can be used to evaluate whether wildlife populations have been adversely affected by EDCs (3, 5, 9). In general, these criteria have been based on epidemiological approaches used to establish cause and effect linkages in an ecological context (10). One of the specific tasks here, relative to other forms of ecological assessments, is that individual and population level effects should be causally linked with effects on endocrine physiology. The criteria put forth by Ankley & Giesy (3) in their weight of evidence approach include:

1 documentation of effects in individuals,

2 documentation of adverse effects in populations,

3 coherence between effects observed in populations versus those in individuals,

4 identification of a plausible mechanism of action consistent with effects in individuals,

5 positive identification of specific contaminants operating through this mode of action,

6 reasonableness of the exposure metrics in terms of individual-level effects occurring via the mode of action under consideration, and

7 evidence of the recovery of populations or individuals upon removal of the chemical stressor.

While these criteria are robust they are not all encompassing. Evaluation of populations suspected of being affected by EDCs should incorporate the epidemiological criteria (time order, strength of association, specificity, consistency on replication and coherence) which form the backbone of

establishing cause and effect relationships as described by Fox (10). There is uncertainty as to how many of these criteria must be met before a response is defined as being due to an EDC. Some have argued that it is the documentation of an effect rather than a mechanism of action which is the significant outcome of studies with wildlife.

EXAMPLES FROM CASE STUDIES

This section provides a summary of several case studies describing physiological changes in wildlife which have been attributed to EDCs (Table 1). This is not intended to represent an exhaustive or comprehensive description of all such studies but rather ones which fulfil many of the criteria listed above by Ankley and Giesy (3). More comprehensive reports of alterations in reproduction and development in wildlife including those attributed to EDCs, have been summarized elsewhere (11–14).

Table 1. Selected examples of reproductive and developmental abnormalities observed in wildlife that have been attributed to EDCs

Species	Site	Observation	Contaminant	Refs
Invertebrates Gastropods	Marine Estuaries	Pseudohermaphrodism, imposex, intersex, sterility, population declines	Tributyltin	3, 15
Fish Rainbow Trout Roach	England	Increased vitellogenin in males, altered testis development	Sewage effluent	20, 21, 22
Lake Trout	Great Lakes	Early life stage mortality mortality; deformities; blue sac disease	Dioxin and related AhR agonists	16, 25, 26
White Sucker	Jackfish Bay, Lake Superior	Reduced sex steroid levels, delayed sexual maturity, reduced gonad size	Bleached kraft pulp mill effluent	29, 31
Flatfish	Puget Sound	Decreased hormone levels, reduced ovarian development, reduced egg/larvae viability	PAHs	35, 36, 37
Reptiles Alligator	Lake Apopka	Decreased phallus size, abnormal gonadal development	DDE	38, 39, 40
Birds Waterbirds, Raptors	Great Lakes (Global)	Egg shell thinning, mortality	DDE	41,42
Waterbirds, Raptors	Great Lakes	*In ovo* and chick mortality, growth retardation, deformities	PCB and AhR agonists	41, 43, 44
Western Gulls	California	Abnormal mating and nesting, behaviour, supranormal clutch size, skewed sex ratios	DDT and its metabolites	12, 45
Mammals Mink	Great Lakes	Steroid and thyroid hormone changes, population decline	PCBs and dioxins	17, 47, 48

One of the better examples of population level effects of EDCs in wildlife comes from studies of the masculinizing actions of tributyltin (TBT) on reproduction in marine gastropods (3, 15). TBT originating from antifouling paints used to treat boat hulls has had widespread effects on reproduction in a variety of gastropods. TBT induces a form of pseudohermaphroditism termed imposex in female neogastropod molluscs which is the imposition of male sex organs (penis, vas deferens) and can lead to sterility. A related condition termed intersex occurs in littorinid gastropods and involves TBT-induced alterations of the oviduct which in advanced stages involves splitting of the oviduct and formation of a prostate gland. The underlying mechanism responsible for the actions of TBT are not fully understood although many of the masculinizing effects can be mimicked by testosterone or blocked by testosterone receptor antagonists (15). TBT acts by blocking aromatase activity and / or the activity of sulphur conjugating enzymes which promote the excretion of testosterone and its metabolites. Both of these effects would contribute to an elevation of testosterone levels. The use of TBT has been associated with population

declines and its subsequent ban in many countries has been related to a decrease in environmental levels, a decreased incidence of imposex and gastropod population recoveries.

As a group, fish have been studied more than any other animal taxa in terms of considering endocrine disruption in the field. The majority of these studies have focused on reproduction and development as the primary endpoints (9, 13, 16, 17) and examples of these will be discussed. There is also evidence that thyroid hormone and corticosteroid hormone systems are affected in feral fish populations (16, 18, 19). In these latter cases, there is little evidence that these responses are due to environmental chemicals as opposed to other stressors (e.g. environmental factors, habitat change and diet). Furthermore, the significance of the responses at the individual and population level is poorly defined.

Some of the most compelling evidence for effects of environmental EDCs comes from studies examining fish downstream of sewage treatment works in the United Kingdom (3, 20, 21). This research was initiated in response to observations of hermaphroditic fish in sewage treatment lagoons. An extensive series of studies using caged fish showed that exposure to effluents was associated with massive increases in plasma vitellogenin levels. Elevation of vitellogenin synthesis is diagnostic of exposure to oestrogenic compounds. Subsequent studies suggest that natural oestrogens, synthetic oestrogens from birth control pills and chemicals with oestrogenic activity (alkylphenols) present in the effluent may contribute to these responses (20). At this time, evidence of a linkage between exposure to oestrogenic substances and adverse population level outcomes in fish is much weaker. However, there is evidence that there is decreased testicular growth in caged rainbow trout concomitant with vitellogenin induction (22). Recent observations of elevated vitellogenin levels in fish at selected sites in North America (23, 24) raise the possibility that the risk posed by sewage treatment effluents may be widespread.

There is convincing evidence that exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related aryl hydrocarbon receptor (AhR)-active compounds have contributed to blue sac disease and early life stage mortality in Great Lakes salmonid populations, particularly in Lake trout (16, 25, 26). Blue sac disease is characterized by yolk sac oedema, regional ischaemia, haemorrhaging, craniofacial abnormalities and mortality early in larval development. Induction of the blue sac diseases has been replicated in the lab using pure compounds and fish extracts (25). Recent studies have shown reductions in the incidence of blue sac disease and increased recruitment associated with the decline in dioxin equivalent concentrations in the Great Lakes. The mechanism mediating this effect is thought to involve activation of the AhR battery of genes involved with oxidative metabolism and oxidative stress which ultimately includes effects on apoptosis in endothelial cells (27). While the actions of the AhR agonists in this disease are unequivocal, there is little evidence that these effects are mediated through alterations in endocrine physiology as opposed to direct toxicological effects. .

Alterations in endocrine homeostasis and reproductive fitness have been reported in a variety of feral fish populations exposed to pulp mill effluents (28–31). Studies on the white sucker collected at Jackfish Bay, Ontario, Canada demonstrated that pulp mill effluent reduced gonad size, egg size and fecundity, delayed sexual maturity and altered endocrine function through effects at multiple sites along the pituitary-gonadal axis (29, 31). Recent studies have demonstrated that process changes within the pulp mill can alleviate many of the endocrine and whole organism responses but the identity of the EDCs are still unknown. β-sitosterol, a plant derived compound present in high quantities in pulp mill effluents, can cause steroid hormone changes similar those seen following exposure to pulp mill effluents but it is unlikely to be solely responsible for those changes (31, 32). Female mosquito fish exposed to pulp and paper effluent from a Florida mill were masculinized presumably due to either aerobic / anaerobic bacteria transforming β-sitosterol into an androgenic hormone (33) or by the actions of stigmastanol (a related compound) and its degradation products (34). Further work is needed to confirm the identity of the EDCs within pulp mills and to evaluate population level impacts.

Polyaromatic hydrocarbon (PAH) exposure has been linked to depression of plasma sex steroid hormone levels and inhibition of gonad development in marine flatfish in Puget Sound (35–37). PAHs have multiple actions including negative effects on ovarian follicle recruitment, spawning and the viability of eggs and larvae. Questions remain as to which PAHs are involved and their mode of action. PAHs may be a more significant EDC than is currently recognized due to localized areas of high PAH

levels across the globe and numerous other examples which suggest a linkage between PAHs and adverse reproductive responses in fish (17, 29).

Amphibian populations have declined globally and a variety of factors including global climate change, increased ultraviolet light levels, habitat loss, disease and the toxicity of agricultural pesticides and industrial pollutants have been implicated. There is little evidence to suggest that amphibians have been affected as a result of alterations in endocrine homeostasis initiated by environmental EDCs although very few studies have considered this possibility (3, 17). Based on the conservation and homology of endocrine systems in vertebrates there is no *a priori* reason why amphibians would be less sensitive to EDCs than other groups (7). Ankley & Giesy (3) have suggested that the retinoic acid system which controls cellular differentiation, morphogenesis and pattern formation may be a sensitive target for the actions of EDCs and account for some of the deformities seen in amphibians throughout North America.

American alligators in Lake Apopka have exhibited decreased numbers of juveniles, decreased clutch viability and abnormalities of the reproductive organs following a spill of the pesticide dicofol and sulphuric acid in 1980 (38). Subsequent studies showed that hatchlings of both sexes have altered gonadal structure, abnormal ratios of the sex steroids 17β-oestradiol and testosterone and that males have reduced phallus size (39, 40). Linking the observed effects with a specific chemical or defining the underlying mechanism responsible for these effects has proved difficult. Interactions of chemicals with the oestrogen and androgen receptor or acting through alteration of steroid hormone biosynthetic / degradation pathways have been suggested but it may be that this is a situation where chemicals are exerting their effects through multiple mechanisms of action (40).

Numerous studies have shown that agricultural chemicals and industrial waste chemicals exert adverse effects on feral bird populations (12, 17, 41). Two major syndromes (egg shell thinning and PCB-induced teratogenesis) have been linked to chemicals that function as endocrine disruptors. As well, the increased incidence of abnormal behaviour in gulls inhabiting regions contaminated with DDT is often cited as evidence of organochlorine-induced oestrogenic effects in birds (12, 41).

The eggshell thinning effect of DDT and its stable metabolite p′p′-DDE may well represent one of the most famous incidents in wildlife toxicology (42). Eggshell thinning has been associated with broken and crushed eggs and breeding failure in a variety of raptors and colonial fish-eating birds. Surprisingly, the mechanism of action of DDE has never been adequately described (41). The shell gland of the oviduct appears to be the primary target for the actions of DDE where it has been implicated in reducing the activity of calcium dependent ATPase and calcium uptake, influencing the binding of progesterone to its receptor and inhibiting prostaglandin biosynthesis (41). While population recovery has been apparent in North America since the ban of the use of DDT, the risk still exists in third world countries where DDT usage remains high.

The PCB-induced syndrome is characterized by relatively normal egg shell development, *in ovo* and chick mortality, growth retardation, subcutaneous and pericardial oedema and deformities of the beak and limbs (43, 44). Gilbertson *et al.* (43) coined the term Great Lakes embryo mortality and edema syndrome (GLEMEDS) to describe these effects which have been observed in a variety of fish eating birds on the Great Lakes including herring gulls, double-crested cormorants, Forster's and common terns and bald eagles. There is strong evidence that chemicals which bind to the AhR including PCBs, PCDFs and PCDDs mediate these effects. The syndrome correlates with dioxin toxic equivalents which in the Great Lakes are primarily a result of accumulation of coplanar PCB congeners (44). The precise linkage between Ah receptor binding and endocrine disruption in mediating these responses are poorly understood. Alterations in retinoic acid and thyroid hormone physiology and increased steroid metabolizing enzyme activity have been implicated. There are questions as to the ecological relevance of GLEMEDS as even though the syndrome is associated with profound effects on individuals, there is an overall increase in the populations of birds affected by these chemicals.

Skewed sex ratios in favour of females, abnormal nesting behaviour including supranormal clutch size and female-female pairing was reported during the 1970 and 1980's in gulls from regions of California and the Great Lakes where organochlorine contaminants including DDT were present in high quantities. The skewed sex ratios were suggested to be related to oestrogenic contaminants which contribute to

either differential male mortality or feminization of male embryos. This was supported by lab studies in which exposure of seabird eggs to DDT, albeit at high concentrations relative to the levels seen in eggs collected from the wild, induced the development of ovarian tissue and oviducts in male embryos (12, 45, 46). However evidence that this mechanism of action was operational in the field is lacking (41).

One of the most widely cited examples of endocrine disruption in feral mammals involves mink populations in the vicinity of the Great Lakes. The number of mink in this area is greatly reduced and feeding trials using Great Lakes fish have led to adverse reproductive effects in ranched mink (3, 47). As the Great Lakes fish contain elevated levels of a number of synthetic organochlorines, including pesticides and PCBs, it has been difficult to determine which specific chemicals are responsible for the adverse effects. The evidence indicates that PCBs and dioxin are responsible for these effects (47, 48), which include changes in uterine oestrogen and progesterone receptors and changes in thyroid hormone levels (17). Nevertheless the linkage between exposure, endocrine alterations and whole animal responses is essentially absent.

A number of other investigations point to associations between environmental contaminants and alterations in the endocrine system. Several studies with seals have implicated organochlorine insecticides and PCBs in adrenal hyperplasia (49) and in alterations in thyroid hormone and vitamin A deficiency (50). These point to a possible link between environmental exposure and endocrine disruption in mammals but further studies are needed to make this linkage. As a final point, there are numerous examples which show that naturally occurring phytochemicals can have profound effects on the reproductive and endocrine function in feral and farmed mammals (8, 51). Whether man-made environmental chemicals with similar properties may affect wildlife in general has not been considered.

RESEARCH NEEDS

In reviewing the case studies, it is apparent that the variety of responses in wildlife that can be attributed to EDCs are most obvious in species inhabiting areas that have received extensive chemical contamination. There are unanswered questions of whether background levels of the chemicals mediating these effects pose significant risks to wildlife or if the range of effects are restricted to the wildlife we have studied this far. There are several research needs that must be addressed if we are to improve our understanding of the risks posed by EDCs. Many of these relate directly to the uncertainties that were introduced earlier in the chapter including the need for increased understanding of the biology and physiology of sentinel species and the ability to interpret the significance of effects at the suborganismal and organismal level in relation to population and community level responses. Risks should be determined by integrating the best available information derived from epidemiological, *in vivo* and *in vitro* approaches and exposure assessments.

An ongoing need is the ability to identify substances that may contribute to effects in wildlife, define their mode of delivery, biological fate, mechanism of action and environmentally acceptable levels. Both the timing and magnitude of exposure as well as changes in sensitivity of target tissues over time are important determinants in defining the effects of EDCs and need to be integrated with fate and exposure data.

The lack of internationally approved and standardized methods of collecting samples from wildlife and the uncertainty of how these are to be interpreted are reasons why data from wildlife studies have often been under-utilized when setting regulatory agendas (4, 52). The future development of test methods needs to focus not only on sampling wildlife but in establishing *in situ* and lab based exposure protocols that investigate both *in vitro* and *in vivo* responses which can be used to evaluate effects and investigate mechanisms of endocrine disruption.

The case studies illustrate that effects of EDCs are mediated at many different physiological levels and it is the diversity of these responses that make predictions of effects so difficult. For example effects of DDE on egg shell thinning or DDT on avian behaviour would not be predicted by current receptor based screening tests that are being used so extensively. A further complication is that EDCs may have multiple effects and many mechanisms of action. Research focused on addressing the mode of action of suspected EDCs and the comparative endocrinology of wildlife species must continue in order to reduce

these uncertainties. Unquestionably it is the diversity of responses that highlights the need for continued wildlife monitoring and the development of *in vivo* testing strategies in evaluating the activity of putative EDCs.

Given the breadth of responses seen in wildlife to date it is imperative that research continues to address the extent of risk posed to wildlife by EDCs. This will necessitate collaboration and cooperation on an international scale to identify populations that are at risk, to dedicate the resources to address critical knowledge gaps and to ensure that the most current and up to date methodologies in the endocrinology, physiology, ecology and epidemiology are available when evaluating effects on wildlife. Finally we need to communicate the results of our studies widely and effectively such that the true risk posed by EDCs are understood and that policy decisions and regulatory practices are based on the best available scientific knowledge.

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