

Topic 4.13

Endocrine disruption in wildlife: The future?*

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Abstract: Probably the only thing that can be said with certainty about the future of this field of ecotoxicology is that predicting it is foolish; the chances of being right are very slim. Instead, it seems to me likely that unexpected discoveries will probably have more influence on the field of endocrine disruption than the outcomes of all the planned experiments. It is certainly true that chance discoveries, such as masculinized fish in rivers receiving paper-mill effluent, imposex in molluscs due to exposure to tributyltin, and feminized fish in rivers receiving effluent from sewage-treatment works, have been pivotal in the development of the field of endocrine disruption in wildlife. I consider that further such discoveries are likely, but I do not know which species will be affected, what effects will be found, what chemical(s) will be the cause, or what endocrine mechanism(s) will underlie the effects. The recent realization that many pharmaceuticals are present in the aquatic environment only underscores the range of effects that could, in theory at least, occur in exposed wildlife. What is somewhat easier to predict is the research that will be conducted in the immediate future, which will build upon what is known already. For example, it is clear that wildlife is rarely, if ever, exposed to single chemicals, but instead is exposed to highly complex, ill-defined mixtures of chemicals, including many that are endocrine active in various ways. We need to understand much better how chemicals interact, and what overall effects will occur upon exposure to such mixtures. We also need to move from assessing effects at the individual organism level, to understanding the consequences of these effects at the population level. Then, we need to determine the significance of any population-level effects due to endocrine disruption in comparison with the impact of many other significant stressors (e.g., over-exploitation, habitat loss, climate change) that also negatively impact wildlife. Such research will be difficult, and time-consuming, and will probably produce many surprises. All I can be fairly certain about is that the next few years are likely to be as interesting and exciting as the last few have been.

INTRODUCTION

While thinking about this chapter, it became apparent to me that I was finding it much more difficult trying to predict the future than I do writing research papers based on results. Many years ago I read that predicting the future was foolish, because unanticipated events usually have a great deal of impact, and influence things in ways that cannot be predicted. At best, knowledge of the past perhaps makes the future less surprising than it otherwise might have been. It is certainly the case that, ten years ago, when I first became involved in the field of endocrine disruption, I had no idea that it would grow as it has,

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to become a major field of biological sciences in its own right. This failure of mine to predict the enormous growth in activity in the field over the last ten years [1] does not bode well for my attempts in this chapter to predict the next ten years. Nevertheless, I will try and use my knowledge and experiences gained over the last ten years to, if not accurately predict future developments in the field of endocrine disruption (the chances of being correct are, I think, very slim), perhaps provide some pointers as to where I think major advances might be made.

Given the history of the development of the field of endocrine disruption, it seems to me likely that unexpected discoveries will probably have more influence on the field than the outcomes of all carefully planned experiments. It is certainly true that chance discoveries, such as the presence of masculinized fish in rivers receiving effluents from paper and pulp mills [2], imposex in many species of molluscs due to exposure to tributyltin (TBT) emanating from antifouling paints used on ships [3], and feminized fish in rivers receiving effluent from sewage-treatment works (STW) [4,5] have been pivotal in the development of the field of endocrine disruption in wildlife. I consider that further such discoveries are likely, but I do not know what species will be affected, what effects will be found, what chemical(s) will be the cause, or what disrupted endocrine mechanisms will underlie the effects. The recent realization that many pharmaceuticals are present in the aquatic environment [6], many of which are intended to alter the endocrine status of the recipient (intended or otherwise), only underscores the range of effects that could, at least in theory, occur in exposed wildlife.

It is probably easiest to predict (or at least try to predict) the immediate future—say the next year or two—less easy to predict reliably the intermediate future—say the next five to ten years, and probably impossible to predict the distant future. Hence, I will begin by speculating on possible developments in the near future, and finish with some more, very speculative, ideas about the more distant future.

THE NEXT FEW YEARS

One thing it is possible to say with a fair degree of certainty is that we will learn a lot more in the next few years about issues that we already know something about, but presently lack detail and depth of knowledge. One of my colleagues recently described this phenomenon as the field getting fatter! We need to do this if we are to put endocrine disruption in wildlife onto a solid foundation.

Let me provide an example. It is now well established that intersex roach (a small member of the carp family of fishes native to much of northern Europe) are widespread in British rivers [5]. In some rivers all of the “males” are, in fact, intersex. Further, intersexuality seems to be associated with the presence of STW effluents. Recent research has demonstrated that intersex fish can also be found in polluted areas of the marine environment [7,8], and that they are not confined to the United Kingdom; intersex fish occur in Spain, [9], Germany, [10], and Italy [11]. Hopefully, research presently underway will better define exactly where intersex fish are found (in what countries, and at which sites in these countries), and how badly affected the fish are. Close scrutiny of the “testes” of these fish, perhaps using modern techniques such as immunohistochemistry and gene arrays, should help not only to define the problem, but also provide information about the mechanisms underlying the intersexuality. Thus, within the foreseeable future I would hope that we will have a much clearer picture concerning the worldwide prevalence of this example of endocrine disruption in wildlife, and perhaps also have a better idea as to exactly what developmental processes are affected, how, and when. This building on our rather flimsy present state of knowledge will provide the more solid foundations required to enable us to progress with more confidence, and in unity. All new areas of research develop in this manner; initially surprising results are built upon (or refuted) by subsequent research, which provides the solid foundation to progress with confidence.

A second example of how the field may progress in the next few years concerns the identification of the causes of the examples of endocrine disruption reported in wildlife. However, I should say that I feel less confident about trying to predict the future of this aspect of endocrine disruption, because I consider it a very difficult one. Generally speaking, it is very difficult to confidently identify the chem-

ical, or chemicals, causing a particular effect on wildlife. This is because a very large number (around 100 000) man-made chemicals are in everyday use, most of which will reach the environment one way or another. Here they will degrade, to varying extents, and at different speeds, to a wide range of intermediate chemicals. We know very little about these processes. Hence, we know little about the exposure of wildlife to chemicals; ignorance outweighs knowledge at every stage of the process.

Occasionally, a specific effect can be closely associated with the use of a particular chemical, greatly aiding establishing cause and effect. Such was the case with imposex in molluscs and TBT, although it still took ten years to link the two. More often, however, circumstances do not allow such links to be established, in which case other approaches need to be undertaken if the causative chemicals are to be identified; such was the case when a toxicity identification and evaluation (TIE) approach was used to show that the main estrogenic chemicals in STW effluents of domestic origin were natural and synthetic sex steroids [12]. However, in other well known examples of endocrine disruption in wildlife, such as the “feminized” alligators in some lakes in Florida, it has proved much more difficult to identify the causative chemical(s). Much more research is needed in this area, if we are to be able to find solutions to the problems that have been identified in wildlife (see later). I predict, and I hope I’m not wrong with this one, that significant advances will be made in this area in the foreseeable future. Perhaps different approaches, such as utilizing bile as a “sink” of chemicals to which the organism has been exposed [13], will aid progress. Chemical analysis of adipose tissue (fat), which as an approach hasn’t been used much yet in studies of endocrine disruption in wildlife, might also pay dividends. Despite the undoubted difficulties, future research must address the issue of causality, because without it little progress in resolving the problem can be achieved.

So, to summarize my views on the immediate future, I think most research (much of which will already be underway) will build upon existing issues within endocrine disruption. Hence, we should learn much more about these existing issues. By this I do not mean to imply that this “follow-up” research will be in any way inferior to that which produced the original findings. It may not be so novel, but it should (and will, I hope) be of high quality. Without it endocrine disruption (with few exceptions, such as imposex in molluscs) will remain on shaky ground, and be open to criticism.

THE INTERMEDIATE FUTURE

Where will we be ten years from now? Of course I don’t know. However, I do feel confident enough to say that technological advances will occur, will be applied to the field of endocrine disruption, and the results gained will shed new light on the field. I will attempt to predict how two or three of these advances (technological and theoretical) will influence the field.

It is very clear to everyone that this is the era of molecular biology. However, molecular approaches have yet to make much of an impression in the field of endocrine disruption (especially of wildlife, partly because of our lack of knowledge of their molecular physiology), although there are recent signs that this position is changing. For example, Nancy Denslow’s group in the United States has begun to study gene expression in fish exposed to endocrine-disrupting chemicals [14]. The rapid spread of microarray technology into comparative physiology (using laboratory animals such as the zebrafish) will certainly allow a quantum leap forward to be made in our understanding of how chemicals affect gene expression. It is likely that tens, if not hundreds, of genes will have their expression altered when an organism is exposed to an endocrine-disrupting chemical. The problem will then be to interpret the multitude of data in a meaningful way, in order to improve our understanding of what adverse effects are caused by chemicals, and what the consequences of these effects are. This area of research, now called toxicogenomics, may well be a major area of interest in the next few years. Presently it is difficult to see how toxicogenomics can be applied to wildlife, when we know little or nothing about the genome of the organisms of interest. However, I may well be surprised by how rapidly advances in technology and our understanding of the genomics of wildlife allow microarray technology to be applied to wildlife, to address fundamental issues about how chemicals in the environment cause changes

in gene expression: Will different chemicals produce distinct “footprints”? It may even become possible to ascertain if persistent changes in the environment (such as the presence of estrogenic chemicals due to input of STW effluents) create a selective pressure strong enough to lead to altered genotypes, a type of environmental genomics. Put another way, is it possible that fish exposed to estrogenic effluent for many generations evolve the ability to be less adversely affected by the effluents, and hence reproduce more successfully, than would naive (unadapted) fish? Such questions may well become answerable, and hopefully answered, in the foreseeable future.

Besides technological advances, there are likely to be theoretical advances that lead to significant advances in our understanding of endocrine disruption in wildlife. One example will probably be improved predictability of the effects of mixtures of endocrine active substances (EASs). As already stated, the environment usually contains not one or two, but many, EASs. Thus, wildlife are almost always exposed to complex (presently usually ill-defined) mixtures of chemicals, including many EASs with various types of activity (estrogenic, antiandrogenic, etc.). How will organisms respond to these complex mixtures, and will it be possible, based on sound scientific principles, to understand these responses of organisms well enough to be able to accurately predict them? If this could be achieved, regulators could introduce combination effects into legislation, and hence better protect wildlife from the effects of EASs.

Recently, biomathematical concepts initially developed decades ago have been applied to the field of endocrine disruption, in collaboration between ecotoxicologists and biomathematicians/biostatisticians. Relatively simple experiments, using binary mixtures *in vivo* [15], and somewhat more complex mixtures *in vitro* [16], have shown that it is possible to accurately predict the effects of these (admittedly fairly simple) mixtures of EASs. Future research needs to expand this work, to include more complex (and environmentally realistic) mixtures, both in terms of the number of chemicals in the mixtures tested, and the variety of endocrine activities. This research cannot proceed without the collaboration of scientists from different disciplines, especially ecotoxicologists, physiologists, and biomathematicians. It will advance not only our knowledge of the effects of mixtures of EASs, but also lead to major advances in the mathematical and statistical principles underpinning the effects and their predictability. Major gains in risk assessment procedures should follow.

The examples above illustrate that, in the future, major advances in our understanding of endocrine disruption may well require the collaboration of scientists with quite distinct areas of expertise. Research in the area of biological sciences has become progressively more multidisciplinary, involving larger numbers of researchers (witness the genome sequencing projects of the last few years), and I think this movement will also be required in endocrine-disruption research if it is to progress significantly. For example, one of the key questions to address is whether the effects observed in individual organisms (intersexuality, impaired or inappropriate reproductive behavior, elevated vitellogenin concentrations, etc.) lead to adverse population-level effects. Probably only in the case of TBT and imposex in molluscs has it been shown that endocrine disruption can lead to population-level effects (local extinctions, in that particular case). In all other cases, we know much less about the consequences of the endocrine disruption. Most often we do not even know if the effects reported are adverse, in the sense that they reduce the fitness of the affected individual (for example, reproduction could be impaired or even prevented). Only very recently [17] has it been possible to demonstrate that intersexuality in fish does lead to adverse effects at the individual level, in this case to reduced fertility. But, this does not help us in assessing whether populations of fish are threatened, which is what we really want to know. We could, of course, sit back and wait to find out, but this would not be a very responsible strategy. An alternative approach is to model the possible consequences at the population level, which would require fish physiologists, population geneticists, chemists, and population modellers to work together to develop and test the predictive models. I hope that in the future such groups of diverse scientists will form, to tackle some of the key outstanding questions. Up until now, only scientists with similar expertise tend to work together, which usually means that only specific, narrow issues within the wider field are addressed.

Funding agencies have recently become aware of this problem of scientists working in isolation and are beginning to use their clout to “encourage” scientists to work together more. I support this movement and predict that it will become much more common for groups (sometimes large groups) of research scientists, incorporating wide ranges of expertise, to form to address a question or hypothesis that cannot be tackled by small projects. Many of the most important presently unanswered questions in the field of endocrine disruption (are there population-level effects; can the effects of complex mixtures be reliably predicted; what are wildlife exposed to, etc.?) will require broadly based approaches if they are to be addressed successfully.

THE DISTANT FUTURE

I said at the beginning of this chapter that it was foolish to try and predict the future, and that the further one projects into the future, the more unreliable (and plain wrong) these predictions are likely to be. Therefore, I do not propose to try and guess where the field of endocrine disruption will be in ten plus years, other than to say that I think the field will not have gone away (as some suggested when it expanded rapidly, sometimes on not much more than hype, in the 1990s). I hope that the field will mature into a major area of research within biological sciences, incorporating such diverse disciplines as ecology, animal physiology, hydrology, molecular genetics, and chemistry. In many respects, endocrine disruption can be seen as the issue that opened our eyes to the unexpected effects that chemicals released into the environment can have on wildlife. It taught us how little is known about what chemicals are present in the environment (especially the aquatic environment), how they behave in the environment, and what effects (if any, of course) they have on our fauna. We are in a state of ignorance, which is why we shouldn't be surprised when unexpected discoveries, such as the worldwide effects of TBT on molluscs, are reported. More will surely follow. Once one realizes that around 100 000 man-made chemicals are in everyday use, and entering our environment, where thousands of species of animals live, most of which have hardly been studied, if studied at all, it is difficult not to conclude that some surprises await discovery. As we learn more about the endocrine systems of organisms (new hormones are still regularly discovered, even in that most studied of species, *Homo sapiens*), it becomes apparent that chemicals could perturb it in many ways. Perhaps in the future the focus will shift from chemicals adversely affecting reproduction to those affecting other physiological processes—most are, after all, controlled by hormones.

Perhaps I can conclude the serious side of this chapter with a note of hope and optimism (after being rather negative so far). As pointed out in Peter Matthiessen's historical perspective chapter (Topic 4.1), many thousands of research papers concerned with endocrine disruption in wildlife have already been published, and many more will surely follow. The vast majority (probably 99 % or more) are concerned, in a multitude of different ways, with describing the problem. Of course this is probably appropriate: we need to understand the problem first and foremost. However, as researchers charged with sustaining the quality of our environment, we have a duty to provide potential solutions to problems, not only just describe one problem after another. I would like to see, in the future (but soon!) more emphasis being placed on finding solutions to the problems associated with endocrine disruption in wildlife. There are encouraging signs that this is occurring already, albeit not to a great extent. For example, improvements in treatment processes at STW would aid the removal of endocrine-disrupting chemicals, and hence reduce the concentrations of these chemicals to which aquatic wildlife were exposed [18]. In addition, other researchers are engaged in developing and applying new technologies to improve the performance of STW, such as the use of titanium oxide catalysts to remove endocrine-disrupting chemicals from effluents [19].

Finally, please do not judge me in ten years time on whether or not I was successful in predicting the future of this field of research. I am not a clairvoyant, and do not believe people can predict the future. However, I look forward to the next ten years of research in this still very young field of science,

and to the many surprises it will undoubtedly bring. I also look forward to that research improving the quality of our environment for the myriad species that live there, so that their lives can also be improved.

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