Research on Endocrine Disruption in the UK

Kathleen Cameron

DEFRA

I would like to thank the organizers for inviting me. It has been a very enjoyable meeting so far, and I hope myself in the last presentation also meets your expectations.

I am going to update you on some of the things I told you about last year. When I was here last year, I was from the Department of Environment, Transport and the Regions. Since our elections in May in the U.K. we have been reorganized and Environment Protection is now in the Department for Environment, Food, and Rural Affairs.

The department that I work in is Chemicals and Biotechnology Division, and our responsibilities relate to assessing the effects of industrial chemicals, pesticides and veterinary new medicines in the environment; that also includes POPs and endocrine disrupters.

I am going to focus on two things in my presentation today. Primarily the research and to update you on where we are on our research programs in endocrine disrupters, and then say something at the end of the presentation about what we are doing in relation to specific chemicals that have been mentioned during the course of the last couple of days.

To remind you of the background: in 1995 our Institute for Environment and Health published an assessment of environmental estrogens, and this came out with a long series of research recommendations. At about the same time, the U.K. hosted the European workshop on the impact of endocrine disrupters on human health and wildlife in Weighbridge at the end of 1996.

Again, at around the same time we established the interdepartmental group on endocrine disrupters. This group is made up of representatives from departments with an interest in funding research in this area. It also has membership from our agencies, and also our research councils, which fund primarily fundamental research.

The interdepartmental group took the recommendations from both of these reports and compared them with the research that was already underway in the U.K. and identified three areas that we felt there were gaps in information about what was happening. These were in male reproductive health, in the marine environment, and in wildlife and ecosystems more generally.

The male reproductive health: the Health and Safety executive, which focuses on the safety of workers, sponsored a workshop at the end of 1996 to focus what our specific needs were in terms of male reproductive health.

Those needs were put out to competitive tender, which is a requirement for all government-funded research in the U.K., and a program was put into place, which started in 1998 and will finish next year. The program is approximately £1.9 million and consists of five epidemiological studies, one completed so far. Two of the studies focus on impacts on sperm quality and two are looking at hypospadias.

The study that has been completed is an examination of the geographical epidemiology of testicular cancer, prostate cancer, and cryptorchidism. It was carried out by a small area Health Statistics Unit, which is a government-funded group, which specifically focuses on examining the links between incidence of disease and point source pollution. This was completed earlier this year, and showed that the geographical epidemiology of these three conditions is not consistent with environmental exposure to chemicals.

That is on the hypothesis that environmental exposure would be changing, so you would expect to see some variation in the distribution of these conditions, which they did not. So I think it is also worth

remembering the points that were made earlier in relation — particularly in the databases — to birth defects.

Moving on to the marine environment. The program called Endocrine Disruption in the Marine Environment was established to address the issues in the marine environment. We felt that we knew very little, so this was quite a broad program to start out with. It is funded by DEFRA, the Environment Agency, and the Scotland and Northern Ireland Forum for Environmental Research, with a contribution from CEFIC-EMSG.

The program started in 1998 and finishes at the end of this year, although there are a couple of small bits of work that have been extended to carry on until spring of next year. This one cost about £1.5 million, and involves five U.K. research laboratories.

I am going to very quickly run through the conclusions with a small amount of data. I think it is fair to say that this is huge program of work and I cannot possibly do justice to the amount of work and effort that went into this, but I will pick out the few highlights.

In terms of examining chemicals, one of the conclusions was that several androgens and estrogens are present in large U.K. estuaries, mainly in sediments, synthetic estrogens appear to be of major biological significance.

On this next slide, you can see the measurements that were made in the different effluent surface waters and sediments, this is summary data from two estuaries. What you can see is that if you look at the effluent surface waters and sediment core waters, you are talking about nanogram levels of estrogen equivalents. If you are looking in sediments, you are talking about micrograms.

Here is analysis of the chemicals present. On this side, the effluents in waters: so 17 β-estradiol, androsterone, DHP, and nonylphenol. Estradiol accounted for 84-90% of the estrogen activity in effluence and waters.

The sediments proved to be much more difficult, and these were the three chemicals that were identified: nonylphenol, cinnarizine, and I am not going to try and pronounce that one. These account for only 1% of the total activity in sediments, and the work to identify the remaining 99% is carrying on at the moment.

A similar picture for androgens: this is summary data from seven estuaries. Again in effluents, surface waters, and sediment core waters, you are talking about nanogram levels, and then in the sediment again microgram levels, this time of DHT equivalents.

I think it is also worth saying that most of the analyses showed very low levels of androgens except for one estuary, or rather one site, and this is the Irving Valley sewer effluent. The Irving Valley is in Scotland, and this is a primary-treated effluent. Analysis of the effluent showed these compounds, all of which are metabolites of testosterone, and they account for 99% of the androgenic activity in that effluent.

Further conclusions: Prof. Depledge mentioned this in his presentation this morning that crustacea do not produce vitellin in response to estrogens.

Work was done on four estuarine species of fish, all of which showed some degree of feminization, so biochemical, cellular, and macromorphological. Experiments with sand gobies in laboratories showed that these changes are associated with reproductive impairment.

Picking out one or two bits from this. One of the observations from the EDMAR program that has not been seen before is in relation to urogenital papillae. This is a structure that sand gobies possess; the females use it to deposit eggs and the males use them to deposit sperm.

The left hand side is a normal male, and you will see it is smooth. I do not have a picture of the female, but in the female the end has got lots of villi at the end, and you will see on the right hand side a sort of intermediate structure, where there appear to be some villi forming at the end.

In the laboratory, it was shown that exposure to estradiol can induce this condition. The light bars are after 13 weeks of exposure and the darker bars after 22 weeks of exposure. In samples collected from the field, these changes to the urogenital papillae were also observed, but I do not have a slide to show that to you.

Despite all the work we are still not clear on the implications for fish populations, and I think that is one of the things that you will see later on that has come out as a future priority.

One other thing I would like to mention that was done through the EDMAR program was the development of an assay for spiggin in sticklebacks. The person who did all this work is Dr. Ioanna Katsiadaki, and she is in the audience. I am going to give you a very rough overview, but if anybody wants more details I am sure she would be delighted to provide them.

Spiggin is produced by male sticklebacks. It is a nest building protein and it is produced under androgen control. If you expose female sticklebacks to androgens you can induce production of this protein.

These two slides show what happens to females on exposure to, I think, DHT. Increasing concentration is along the bottom, and this is after three weeks of exposure, and that is after five weeks of exposure.

The two charts here show that not only can we produce it in the laboratory, but you can get responses in the field. This shows the results from sticklebacks caged in five estuaries, and you can see a slight response in this one which I think is the Teeds.

On this side is what happens to spiggin induction in sticklebacks exposed to pulp mill effluent. You can see here a large response.

Moving on to the work on wildlife and ecosystems. Six priority areas were identified in this program and I will just quickly run through what we have done on each of them.

The first was investigation of significance of endocrine disruption in wild fish at the population level. This was primarily in fresh water. There was a series of projects funded primarily by our environment agency, DEFRA, NERC, which is the Natural Environment Research Council, and the water industry.

This program has been running for quite a long time, since 1995. Some of you may remember that Dr. Jobling presented a lot of the information from this work at this symposium last year.

A summary report is currently being finalized and it should be published early next year.

The conclusions from this work are impacts on several species of freshwater fish, this includes ovotestis, feminization of reproductive tracts in males, vitellogenin induction, spawning asynchrony, and decreased gamete viability. A second conclusion was that there are impacts of varying severity in a range of locations related to the concentrations of sewage effluent.

The chemicals identified as being responsible were ethinyl estradiol, estradiol and estrone, and then alcohol phenolethoxylates and their breakdown products.

A further conclusion was that additive activity could be detected at environmental concentrations, and perhaps importantly, at levels below the no-effect concentrations for individual chemicals. Even though we have done all this work, there are still question marks about the impact on fish populations.

The second priority area was the development of chronic tests. Our ministers felt that the testing work was primarily an area for industry, and we have strongly encouraged our industry to take an active role.

We want to see this work done through the OECD/EDTA work that Herman described yesterday, but we felt that it would be useful to fund some of the background information, which would help interpretation of the tests. So one of the things that we have funded is a very careful analysis of gonad development in fathead minnows.

A further priority area was a better understanding of the environmental fate and behavior of steroid hormones. A jointly funded project is being carried out by the Institute of Hydrology at Wellingford. This work is completed and is being reported at the moment.

Their conclusions were that it was possible to develop models to predict what will happen to steroid hormones, and their intention is to take that model and carry out some further validation.

A further area was investigating recovery of *Mytilus edulis*, and other near-gastropods following controls in the use of tributyl tin. We have carried out a survey around harbors and marinas. The findings from that were that there is a recovery and decreased concentrations, except for areas impacted by shipping. We anticipate commissioning new surveys in relation to the IMO ban, which was agreed earlier this year.

Two further priority areas were investigating top predators and invertebrates. We have initiated a new program of research, relatively modest compared with the others, funded by DEFRA (by ourselves), and also by our marine and pesticides colleagues, the National Assembly for Wales, and DAE, Northern Ireland. This started earlier this year; it is in the very early stages.

These are the projects that have been funded under the top predators: an avian project, top predator fish, and then some work in mammals: seal and otter populations in the U.K.. Invertebrates: some work on crustacea following on from the findings in the EDMAR program, some work on terrestrial invertebrates, and also honeybees, and finally some work on nuclear hormone receptors in marine invertebrates.

This last piece of work is yet to start and we anticipate it to start in the new year. There is also an additional study, which is not mentioned there, looking at mollusks and biomarkers of endocrine disruption in mollusks.

With the work on the aquatic environment, on fish, coming to a close, we felt it was time to think about what we had done already and what do we need to do in the future. We organized a workshop in Weymouth in September of this year and we were very fortunate to have a number of international participants, including several Japanese experts.

The group considered three areas, and these are the priorities that they came out with. They are wider research issues: you can see that the population level effects came out as being very important; the relationship between endocrine disruption and other areas of toxicity that was mentioned this morning, endocrine disruption in invertebrates with a focus on the field work, and more work to understand causal agents, sources, and roots of exposure.

The second area was testing. The testing group were much more wordy than everybody else in their recommendations as you can see from this: screening methods to include other endpoints, and then a framework to including these; development and standardization tests for aquatic invertebrates (and this should focus on groups where there is lots of information and important groups in ecosystem functioning); and then benthic and pelagic invertebrates. They want to look at both biomarkers and apical endpoints.

Predictive modeling for mixtures; further work to develop the stickleback work that was carried out under EDMAR; perhaps the development of a cell line which could be used for androgens and anti-androgens; investigation of unusual deaths response curves, and then how do we integrate those into the risk assessment process.

This is can we make use of existing data and modeling to establish the relevance of the Tier 1 and Tier 2 testing endpoints that have been described earlier in the meeting, and integrate those into risk assessment, and then finally the developments of QSARs.

The third area that was considered was what do we need in terms of monitoring. The monitoring group thought we needed better spatial surveys to look at trends, and a focus on SACs, which are special areas of conservation, which are designated under European legislation.

There was a lot of support for a thorough investigation of a single catchment. The idea would be to use all the tools that we have now and perhaps some of the new tools that are being developed, and get a very good picture of what is happening.

Also, discussed were an improved understanding of ecosystems to improve risk assessment, and then finally the development of new tools and techniques to develop chemicals and biological effects. Discussed for biological effects were some of the things that we have not looked at in much detail, or some of the species that we have not yet focused on.

The intention is that the funding departments will take these recommendations and look at them against their policy needs, and we will begin a new program of research starting in the new financial year, which is April 2002.

I want just to finish with a little bit on what we are doing on specific chemicals. We do not fund for government-funded research with policy in mind; in our case it is what do we need to do in terms of protecting human health and the environment from risks and chemicals. I have just picked one or two chemicals that have been mentioned during the course of this meeting.

Nonylphenol: under the European chemicals legislation there is a process for assessing risks, and then when risks are identified, development of a risk reduction strategy and then taking that forward into legislation. With nonylphenol — this is a U.K. led assessment — the assessment was actually published a couple of years ago, the risk reduction strategy was published a couple of months ago, and on the back of that we expect proposals for EU legislation by the end of this year.

I guess many people will know that there have been concerns about nonylphenol for a very long time and we have had voluntary action within the U.K. to move away from many uses.

Tributyl tin antifoulants: we have legislation on use of these antifoulants on small boats. You will know that the IMO convention agreed in London in October 2001 banned application on ships by January 2003, and then bans complete use by January 2008. On the back of that convention we are expecting legislation before the end of this year.

Bisphenol A: UK is again leading on this. The draft risk assessment has been carried out, and this identified a number of uses causing problems to human health, and this is primarily to workers and the environment. The assessment ran into difficulties on the endocrine disruption endpoints; for both the human health and the environment they have concluded that further research will be needed. Discussions are underway at the moment as to exactly what that will be and how that will be carried out, what laboratories will do the work, and so forth.

We are going to develop, or begin to develop, the risk management strategy based on the uses identified as causing problems. This work will look at all the uses of bisphenol A, so that should the additional work on endocrine disrupters flag off any problems, we will be in a position to know what to do quite quickly.

We have heard about dioxins, furans, and PCBs. The European Commission published a strategy in October of this year, and it was discussed by the European environment ministers last week. We will be publishing a strategy in the U.K. in the very near future. The U.K. last published a strategy document in 1989, so this revised document will look at the impacts of the measure we have taken already. It will examine changes in the science and understanding, and then make proposals for consultation on what we need to do in the future.

To show you very briefly what happened to dioxin emissions in the last 10 years. This is 1990. I am not sure you can read that, but total emissions to air in 1990 were just over 1,000 g of ITQs per year, and you see that the major source here was municipal waste incineration.

If you compare that with emissions to air in 1999, current emissions are about 350 g ITQs. Municipal based incinerators are now 1% of total emissions. Other sources are accidental fires and open

burning and other sources, which are now much more important. Our new strategy document will be making proposals about how we might deal with those.

Finally, a very brief update on what is happening with the EU chemicals policy. The EU strategy was published in February. The Environment Council ministers considered this in June 2001 and made a series of recommendations.

These have been taken forward through a series of working groups, which started in October 2001 and will carry on working, probably for the next couple of months. The original timetable was that there would be proposals for new legislation in Spring 2002, but given that the working groups have not finished yet, that is probably a bit optimistic.

The sorts of issues that have got to be resolved are what are the information requirements for chemicals, especially at low production volumes, and criteria for authorization. The new strategy contains a proposal that the most hazardous chemicals will be authorized, so they will only be used for certain uses. How that procedure will actually work is still under consideration.

The sorts of chemicals that have been proposed for inclusion are PBTs, CMR chemicals — very persistent and very bioaccumulative — and endocrine disrupters, when test methods are available. The other area that needs thinking about is what to do about products.

I have given you a quick run-through of what is happening. Thank you very much for listening.

Q&A

Takei: Thank you. We are a little bit behind time, we have time for one question.

You have shown the data of the sediment soil, which seems to be relatively high in concentration. Are you trying to do further work on this sediment soil?

Cameron: I'm sorry?

Takei: Sediment soil. You have shown the data of the sediment soil and the concentration was high. Are you planning further work on this?

Cameron: Yes, we are. One of the difficulties has actually been getting the chemicals off the sediments to identify them. That raises the question about whether they are bioavailable or not. We are actually thinking about what is the best way of addressing that problem: would it be better to do some studies looking at bioavailability rather than focusing on trying to identify the chemicals.

Takei: Thank you.