

Disruption of the thyroid hormone system by brominated flame retardants

In vivo toxicity testing in rats is one of the tools to study endocrine disruptive actions of brominated flame retardants within the theme 'human toxicity' under the FIRE project. Four different compounds were selected on the basis of their endocrine potency in *in vitro* reporter systems (see CREDO newsletter issue 4), representativity, production volume, and environmental or human exposure data. These compounds were: tetrabromobisphenol-A (TBBPA), hexabromocyclododecane (HBCD), a commercial "pentamix" and a "decamix", containing mainly penta- and deca-bromodiphenylethers, respectively. All compounds were tested in a sub-acute (28-day) exposure protocol, the former two also in one-generation studies. A multitude of endpoints was evaluated, with a major focus on endocrinology.

Impacts on a whole organism

From the studies with TBBPA and HBCD it appeared that there were some marked effects on the thyroid hormone system.

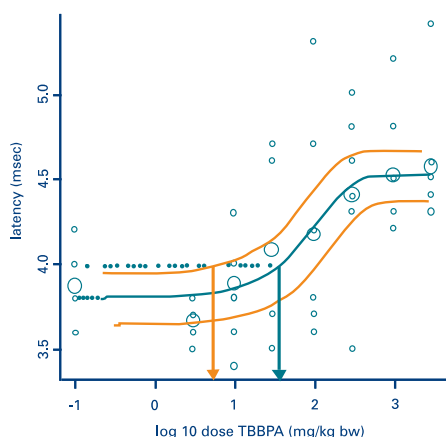


Figure 1: Dose-response curve of auditory latency at low frequency, in male F1 rats of a one-generation study with TBBPA. Large circles are average per dose group. Green arrow: critical effect dose at 5% effect level. Orange arrow: benchmark dose at the corresponding lower confidence level.

In the study with TBBPA, the biologically most relevant effect was probably the increase in latencies of auditory responses (tested with brainstem auditory evoked potentials (BAEP)), in particular, in the lower frequency range (figure 1). This effect is, most likely, related to impairment of the development of the

Continued on page 2

Brominated diphenyl ethers appear in people at increasing concentrations

Results from human exposure assessment

Brominated flame retardants (BFRs) save lives by reducing the flammability of a wide variety of commercial and household products. Despite their societal benefits, some brominated flame retardants are migrating from the products in which they are used and are entering the environment and people. Within the FIRE project, human exposure has been assessed by analysis of BFRs in breast milk and serum from general populations in the Czech Republic (CZ), The Netherlands (NL) and Norway (NO).

In breast milk collected in 2003/04, median concentrations of the sum of tetra- to heptabrominated diphenyl ethers (PBDEs) were 1.68, 3.48 and 2.34 ng/g lipid for CZ, NL and NO, respectively. The congener pattern of the PBDEs was similar in all three countries with BDE-47 being the dominating congener followed by BDE-99 and BDE-153. For Norway the body burden of PBDEs was calculated to be approximately 1 µg/kg b.w. which is about a factor of 40 lower than that calculated for the sum of seven PCBs. However, a skewed frequency distribution of PBDE-levels is observed with about 5% of the subjects having an excess load of PBDEs five times higher than the median. Analysis of archived breast

Continued on page 2

In this edition

- 1 Disruption of the thyroid hormone system by brominated flame retardants
Brominated diphenyl ethers appear in people at increasing concentrations
- 3 Food contamination by androgenic/antiandrogenic compounds
- 4 Environmental impacts of plasticisers
Risk perception and endocrine disruption
- 6 Metamorphosis of the sea urchin
- 8 Research news and meetings
Obituary – Josephus Gerardus Vos

Disruption of the thyroid hormone system by brominated flame retardants (Continued from page 1)

upper (apical) part of the cochlea, the spiral cavity of the inner ear. TBBPA related changes in plasma thyroid hormone levels, including a decrease of thyroxine (TT4), and an increase of triiodothyronin (TT3; only females), are likely behind the impaired auditory responses, because it is known that disturbances of thyroid hormone levels in the neonatal period can result in similar effects. A tentative explanation for these results is that TBBPA competes with thyroid hormones on thyroid hormone plasma binding proteins (mainly TTR in the rat), thus releasing these hormones for metabolism. This explanation is plausible in view of data from the *in vitro* reporter assays, showing a very potent competition of TBBPA with human TTR. The benchmark dose (BMD-L) at the lower confidence level for this effect was in the range of 2 to 28 mg TBBPA / kg body weight per day at specific effect levels for each end point. The 28-day study with HBCD showed consistent effects on the thyroid hormone axis, including decreased T4, increased pituitary and TSH in this gland (by immunostaining), and increased thyroid weight and activation (histopathology, figure 2) mainly in female rats. Some further effects can be understood as secondary to the disruption of the TH axis: increased plasma cholesterol, decreased plasma glucose, and increased bone density measured in collaborative action with the BONETOX project). In contrast to TBBPA, the effects with HBCD are probably indirect, through activation of metabolising enzymes in the liver (e.g. T4 glucuronyl transferase), also indicated by the increased liver weight in females. Benchmark doses at the lower confidence level for these effects were in the range of 20 to 90 mg HBCD /kg bw, for each endpoint at a specific effect level (range 5 to 20%).

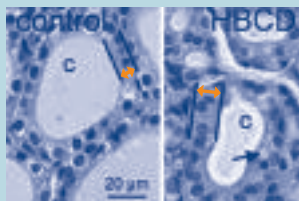


Figure 2: Activation of thyroid gland cells after exposure to HBCD. Cells have increased size (height, orange arrows) and larger nuclei, indicating increased synthetic activity.

It is thus evident that brominated flame retardants can disrupt the thyroid hormone system in a whole organism. These and other results from these studies will now be entered in the theme "integrated risk assessment" of the project, where they will be compared with exposure levels of these compounds, which were measured in humans (plasma and milk), human food, and wildlife. In this way, it should be possible to determine the relevance of the findings in the toxicity studies for health of humans and other mammals.

Leo van der Ven National Institute for Public Health and the Environment, Bilthoven, the Netherlands

Aldert Piersma National Institute for Public Health and the Environment, Bilthoven, the Netherlands

Hellmuth Lilienthal BGFA, Research for Health Protection at Workplace, Ruhr University, Bochum, Germany

Timo Hamers Institute for Environmental Studies, Vrije Universiteit, Amsterdam, the Netherlands

Helen Håkansson Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

Brominated diphenyl ethers appear in people at increasing concentrations (Continued from page 1)

milk and serum samples from Norway show that the body burden of PBDEs has been strongly increasing since the early 1970s to the late 1990s.

This is in contrast to the decreasing levels observed for chlorinated persistent organic pollutants like DDT, PCB and dioxins in breast milk during the last decades. However since the late 1990s, the PBDE body burden seems to have stabilised or slightly decreased. This might be explained by a voluntary discontinuation of the production and use of the technical penta- and octa-BDE mixtures in Europe.

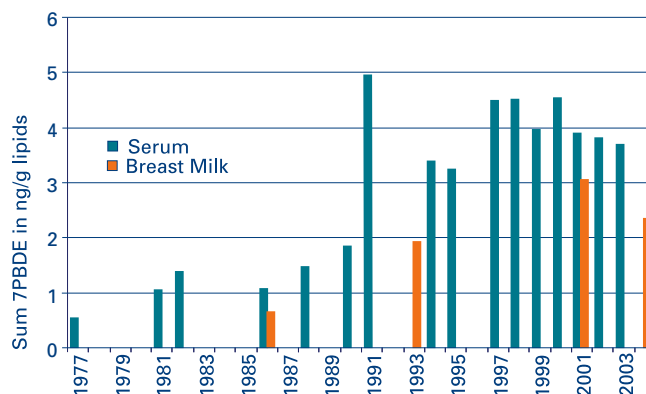


Figure 1: Sum tetra- to heptabrominated diphenyl ethers in pooled serum and breast milk from Norway through three decades.

Similar median concentrations of PBDEs in breast milk (approximately 2 to 7 ng/g lipid) have been found in other European countries. In contrast, median PBDE breast milk levels reported from North America for recent years are about a factor of 10 to 20 higher.

Results for other BFRs in human samples are scarce partly due to analytical difficulties or very low levels. Tetrabromobisphenol A (TBBPA), the BFR with the highest production volume, has been found at concentrations between < 0.1 and 1.0 ng/g lipid in pooled serum samples from Norway. Also for hexabromocyclododecane (HBCD) and the fully brominated diphenyl ether, BDE-209, only few data are available. Preliminary investigations show that HBCD appears in breast milk at concentrations similar to BDE-153. In contrast, BDE-209 has been observed in Norwegian pooled serum samples at levels exceeding those of the sum of the tetra- to heptabrominated diphenyl ethers.

In conclusion, there are indications that the recent voluntary and regulatory discontinuation of the use of the technical penta- and octa-BDE mixtures in Europe has led to a stabilisation of human exposure to these pollutants. However, data on human body burdens of other BFRs including BDE-209 are incomplete and need further investigation.

Georg Becher and Cathrine Thomsen

Norwegian Institute of Public Health
Oslo, Norway

Food contamination by androgenic /antiandrogenic compounds in the European Union

A market basket approach

The food market is a growing and very profitable economic sector. An unavoidable necessity is the application of pesticides in order to improve agricultural production. However, consumers are increasingly concerned about food contaminants; and pesticide residues feature high in their list of worries. For these reasons food monitoring and human exposure data should be used to illustrate what regulators are doing to ensure that the food we eat is safe. An attempt to identify the potential exposure of human populations to androgenic/antiandrogenic compounds (AACs) originating from food is carried out within the COMPRENDO project (Comparative Research on Endocrine Disrupters – Phylogenetic Approach and Common Principles focusing on Androgenic/Antiandrogenic Compounds). It is imperative to obtain detailed information on the extent of AAC contamination in human food from different European areas.

Representative market baskets - considering regional preferences of nutrition as described by the GMES/Food Program (Global Environment Monitoring System, Food Contamination Monitoring and Assessment Program) at the World Health Organization (WHO) - were sampled on three different occasions annually, from local markets in ten European countries (Denmark, France, Germany, Greece, Italy, Netherlands, Poland, Spain, Sweden and the United Kingdom). This offered the opportunity to analyze the different food sources for AAC residues and to identify particular types of food that contribute to higher human AAC exposure. The selected food commodities included: cereal/wheat flour, potatoes, chicken eggs, apples/peaches, butter/oil, meat, wine/beer, orange juice/strawberries, carrots/tomatoes, cheese, fish and shellfish.

The selected compounds, considered to be on the EU priority list for endocrine disrupters, included a range of endocrine active pesticides and their metabolites such as the androgenic fungicide fenarimol (an aromatase inhibitor) and the antiandrogens vinclozolin, linuron and diuron. The metabolites of phenylurea herbicides (DCPU, DCPMU, 3,4-DCA), suspected of endocrine disrupting activity were also analyzed.

The presence of pesticide residues was confirmed in various food commodities such as carrots, potatoes, apples as well as orange juice. Average annual concentrations ranged between 2.5 and 60.7 µg/Kg on fresh weight basis, while some violations of the maximum residue limits (MRLs) were observed in orange juice and carrot samples. Vinclozolin and the metabolite 3,4-dichloroaniline of the phenylurea herbicides (linuron and diuron) were detected in orange juice, while linuron and one of its metabolites (DCPU) were positively identified in potatoes and carrots, together with vinclozolin. Fenarimol was detected in apples.

Dietary intake and risk assessment

Analytical results show that approximately 19% of food consumed in the EU contains measurable residues of pesticides or of their metabolites and that approximately 1.4% contain residues above the MRL.

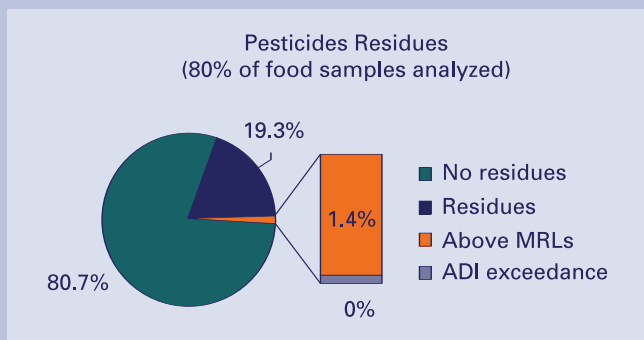


Figure 1: Residues of AAC pesticides in food samples in the EU (Market Basket Approach).

The MRL is that expected to occur in a commodity following the application of pesticides based on good agricultural practice (GAP). It should be noted that MRLs are not a safety parameter because toxicological considerations are not taken into account. In some cases, high MRLs are also established (when GAP trials data are not available) based on the analytical limit of determination of the molecule. From the human health point of view, there is perhaps good reason to accept the “acceptable daily intake” (ADI), the intake of each day and of an entire lifetime, as a relevant safety parameter. If the MRLs are exceeded, comparisons of the mean dietary intake of the exposure with ADIs (long-term exposure/lifetime) and/or acute reference doses (ARfDs; short-term/single day) will then indicate whether there is a possible chronic or acute health risk, respectively.

Two key pieces of information are required to estimate the intake of a chemical present in food. One is chemical residue data and the other is consumption data of foods that could contain the chemical. Residue data provide information on the occurrence and concentration of contaminants of interest in foods. Consumption data provide information on the quantity of each food consumed by an individual consumer or by the total population. Both sets of data can be combined using one of several approaches to estimate the intake of a particular food chemical. Estimated daily intake (calculated as the occurrence mg Kg⁻¹ x consumption g day⁻¹ / 60 Kg body weight) ranged between 0.11 and 4.3% of ADI values. In other words, despite the violations of MRLs, in no cases have pesticide residues exceeded the ADIs and presented a risk to the consumer. With respect to the risk assessment, on the other hand, ADIs are based on single pesticides, however unfortunately our diet contains complex cocktails of pesticide residues and there are no data allowing us to decide which effect they might have on the consumer in the long term.

Vasilios Sakkas, Vasiliki Boti, Vasiliki Valsamaki and Triantafyllos Albanis

University of Ioannina, Department of Chemistry, Greece

Dysregulation of endogenous steroid metabolism potentially alters neuronal and reproductive system development: effects of environmental plasticisers

Environmental impacts of plasticisers

Environmentally-derived plasticisers (EPs) are toxicants which may disrupt endocrine systems in man by altering the metabolism of steroid, thyroid and other hormones, their receptors and cell signalling pathways. As these receptors occur in the brain, the reproductive system and a wide variety of other tissues, EPs are likely to affect cognition, neurodevelopment, and reproductive function.

The central aim of the ENDOMET project is to understand the actions of EPs with the ultimate goal of determining the possible long-term effects to human health. As part of our research, EPs which are known developmental or reproductive toxicants (showing teratogenic and feminising effects at low levels in rats and fish) were studied in human and animal brain, breast, ovary, testicular and thyroid cells. The EPs studied (in low doses, individual chemicals and mixtures) included bisphenol-A, alkyl phenols, and adipate and phthalate esters, all common environmental and food chain contaminants.

Effects of EPs hormone action were studied using reporter gene assays based on oestrogen, androgen, and thyroid receptors. Cell signalling pathways investigated included MAPK, CREB, P-I-3 kinase, NF-kB pathways. The effects of EPs on reproductive potential (oocyte maturation, FSH, LH, EGF receptors) were evaluated using porcine ovarian follicular primary cultures. Hormone and co-factor synthetic and catabolic enzymes included aromatase, sulphotransferases, sulphatase, cysteine dioxygenase, and the sodium-iodide symporter. Early results from ENDOMET about these effects are published in *Molecular and Cellular Endocrinology*, Special Issue on Endocrine Mimicry and Disruption: Plasticisers and Other Environmental Chemicals (Volume 244, issues 1-2, December 2005).

Predicting toxicity

One of the aims of ENDOMET has been to develop *in vitro* tests (incorporating genomic/proteomic approaches) to predict toxicity, which will provide information more relevant to impacts on man, potentially reducing the use of animal testing. At present, there are no specific guidelines in place for the detection of EPs. Our early results using *in vitro* proteomics hold promise of helping to predict how EPs interact with human reproductive and neuronal systems. Easy-to-use biomarkers for EPs and an improved understanding of their potential for toxicity will, hopefully, eventually reduce environmental contamination by allowing plastics manufacturers to test new materials for endocrine disrupting activity and thus provide the public with non-toxic alternatives.

David Ramsden and Nahid Turan

University of Birmingham, UK



Risk perception and endocrine disruption

Results of the ENDOMET workshop

When ENDOMET was set up there was originally a work programme on risk perception by the general public about plasticisers and the possibility of endocrine disruption. However, our initial studies showed that, at that time, the general public had no understanding of endocrine disrupters (EDs) so we moved on to using consumer focus groups who were better informed. This article summarises the key results we have had from these groups.

We have received comments from consumer panels, workshops and representatives from across the EU and we also held a small risk perception workshop in Birmingham (February 2006) for UK input. This included representatives from UK government organisations and consumer groups.

Birmingham risk perception workshop

The workshop panel heard from Dr Ragnor Pedersen (scientific project manager, CREDO) who presented a detailed overview of the types of adverse human health effects of concern that scientists are investigating in connection with exposure to EDs. He discussed some of the latest research findings and talked briefly about current regulatory concerns. Dr David Ramsden (deputy co-ordinator, ENDOMET) then gave a presentation explaining the mechanisms of action of EDs and pointed out that we do not yet know whether plasticisers are actually harmful to humans. Using results from genome scanning, he highlighted the potential effects of some plasticisers.

There was a vigorous discussion at the end of these presentations and the panel was very supportive of the Prague Declaration on Endocrine Disruption (see CREDO Newsletter Issue 5, January 2006). Members of the panel noted that industry had not welcomed this manifesto although industrialists clearly needed to be fully involved in the discussion and in the related research. The panel generally felt that while there had been many scares about food and health topics, the public had relatively little understanding about endocrine disruption, which was seen as a subtle, serious and long-term hazard. They would be interested to find out what alternatives there are to plasticisers or other compounds used industrially – could less toxic compounds be designed and

used instead? It was further felt that risk reduction at an individual level was not an option, given that plasticisers are so widespread in the environment and there was concern that there was as yet no clear message on the effects of EDs in man.

Perceptions of risk across the EU

Analysis from all the panels around Europe showed that consumer perception of risk depended on the life-stage of the consumer (parent, pregnant, elderly) and on the social group, culture and attitudes of the populations involved. Risk was seen as less disturbing if the activity undertaken was voluntary and where there was a degree of control over the exposure. Risks were seen as more disturbing if they arose from an unfamiliar or novel source (such as genetically modified foods), from man-made chemicals and if they were unfairly distributed with some people benefiting while others suffered the consequences.

Who do consumers trust?

Across the EU, there is some variation in the degree of trust placed in various sources as reliable providers of information on foods and health risks; consumer organisations were most trusted in the UK, Italy and Germany while politicians came very low on every country's list. University scientists were seen as very trustworthy in the UK (ranked 3rd out of 23) but only 11th out of 23 in the EU as a whole. Doctors/health authorities take the top slot for food information in the UK but were ranked 8th out of 23 in the EU as a whole. This means that dissemination of results from research programmes must be carefully targeted and provided by groups seen as reputable in their home countries.

Rosemary Waring

University of Birmingham, UK



Key recommendations on risk perception

What can be done to allay public concern?

Risk communication

It is obvious that plasticisers, together with the POPs studied in the CREDO programme, would fall into the category of 'high risk perception' compounds if the population generally were more aware of their existence. There is therefore a window of opportunity to ensure that public perception of risk is realistic. The panels were all in agreement that scientists should explain what is known and where there are uncertainties. Two-way communication was seen as vital, with honesty about any risks, open public discussions, a commitment to listen to and value other opinions and perspectives and 'transparent' regulatory decisions. It was felt that consumer labelling was not really helpful since few consumers could understand the science or avoid the compounds in the day-to-day environment.

Risk assessment

Many of the members on the panels had expertise in consumer representation within the regulatory frameworks of the UK and Europe. It was felt that most regulatory decisions were made with little public scrutiny of the scientific data. Generally, the panels were all agreed on the need for more research, particularly on mixtures of chemicals, with increased surveillance of environmental contaminants and disease patterns.

Risk management

The panels all wanted to know what was being done to manage risks and to be certain that, if new products were introduced onto the market, this was only done if there was a demonstrable need. The risk/benefit ratio was seen as a major guiding concept, with 'acceptability' being a function of the positive good done by the compounds. There should be open discussion of risks with those who might be affected and of the options for dealing with them. It was thought that the public could cope with the 'mixed messages' presented by work on EDs if they were provided as a broad picture so that people could assess and manage risk for their own personal circumstances.

Risk perception of plasticisers

How do plasticisers fit onto the risk perception scale?

- Like other EDs, plasticisers are man-made complex chemicals rather than natural products and are widely used.
- It is difficult for individuals to avoid any risks that plasticisers may produce.
- The potential health effects may be serious and long-term.
- EDs/plasticisers may affect fertility (a very emotional subject).
- EDs/plasticisers may be particularly toxic to children and pregnant women (both high-concern groups)
- The effects of EDs/plasticisers are unknown and so potentially more worrying.



Metamorphosis of the sea urchin

A multi-signal endocrine regulated event

Metamorphosis of the sea urchin is affected by acetylcholinesterase (AChE) inhibiting compounds (organophosphates belonging to the thionophosphate family), in a dose-dependent way: low doses (10^{-7} to 10^{-8} M) of chlorpyrifos and phentoate enhance metamorphosis represented by both rudiment growth and larval reabsorption in the absence of environmental stimuli, in a similar and synergic way with exposures to triiodothyronin (T3), while acute doses (10^{-4} to 10^{-5} M) cause sudden reabsorption of larval structures and release of immature juveniles. Thus, the hypothesis arises that the organophosphates (OP) compounds may exert interference in the endocrine functions of echinoderms.

The sea urchin *Paracentrotus lividus* presents a freely swimming larva and a benthonic adult stage. The substrate choice is crucial for this kind of organism, because in their final environment they must find suitable food and the presence of co-specific adults. In similar marine organisms, such a choice takes place in response to signals of various natures, either physical, chemical or nervous, evoking as a last step hormone release. Before metamorphosis, the larvae undergo a more or less long exploratory period, up to the moment when they receive the suitable signals from the environment. In the laboratory routine, such stimuli are evoked by adding to the rearing sea water stones or *Posidonia* leaves freshly taken from the natural environment, when the rudiment appears competent for metamorphosis (figures 1, 2, 3).

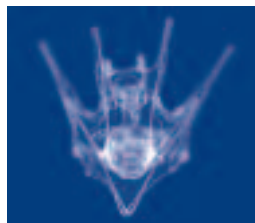


Figure 1: Fifteen days 8 arm larva, courtesy of Andy Cameron and Heinz Gundlach.

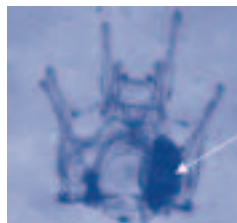


Figure 2: Eighteen days 8 arm larva, with rudiment of juvenile echino (arrow).

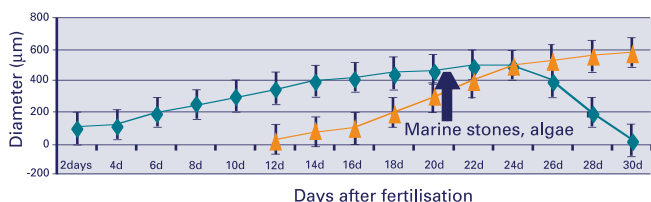


Figure 3: Reciprocal growth of larva (green) and rudiment (orange line). Metamorphosis was enhanced by insertion in the rearing water of marine stones, and algal (*Posidonia*) fragments.

In the absence of stimuli, metamorphosis does not take place (figure 4). Recent work on a diverse array of echinoderm species has demonstrated, as is true in amphibians, that the thyroid hormone thyroxin (T3) accelerates development to metamorphosis. Chino *et al.* (1994) reported the presence of the thyroid-stimulating hormone (TSH) in the

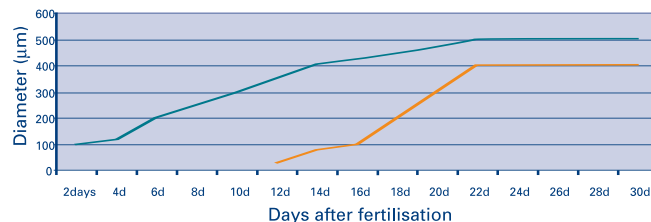


Figure 4: Reciprocal growth of larva (blue) and rudiment (orange line). Without external input (stones, hormones) metamorphosis does not take place.

green algae included in their diet (exogenous source) whereas non-feeding larvae of the sand dollar *Peronella japonica* produce T3 themselves (endogenous source). Thyroxin, one of the iodinated hormones produced by vertebrate thyroids, has been reported to accelerate late larval development in several sea urchins and in the crown-of-thorns starfish (Johnson & Cartwright 1996), and T3 effects on earlier stages of echinoderm development were described by Heyland and Hodin (2004), in the New Zealand sea urchin *Evechinus chloroticus* (Valenciennes). T3 exposure treatment slowed development between the eight-cell stage and assembly of the four-armed pluteus and mid-larval development between the four-armed and six-armed stages, while accelerated progress of eight-armed plutei toward settling, without altering the final percentages of larvae that settled and metamorphosed to juvenile urchins. Such an acceleration of late larval development by T3 may indicate a relatively ancient evolutionary origin of T3 effects on developmental processes (Johnson 1997, 1998; Morse, 1993).

In the past, for other benthonic organisms, such as ascidians (Coniglio *et al.*, 1998) and barnacles (Faimali *et al.*, 2003) we found that metamorphosis may be enhanced by cholinergic signalling. Thus, the hypothesis is advanced that molecules belonging to the cholinergic system may be involved in the regulation of hormone release or activation.

The developing sea urchin larva during metamorphosis was used as a first model to understand if OP compounds may also play a role in the endocrine release/reception, in the course of the project SENSPESTI, aimed at understanding the risk for organismic health represented by exposure to low doses of this chemical family. Our hypothesis was that the chronic exposure to low concentrations of organophosphate compounds, such as those used as neurotoxic pesticides, present in coastal sea water during springtime periods when agricultural lands are treated, could interfere in the signals inducing metamorphosis.

Materials and methods

Embryos and larvae of the sea urchin, *Paracentrotus lividus*, were reared since fertilisation in ultra filtered and pasteurised sea water, fed with micro algae according appropriate protocols, and maintained at 20°C in a temperature controlled room. Metamorphosis was obtained according to standard procedures, by exposing 20 days larvae to the presence of *Posidonia* leaves. Experiments were performed by exposing to tetraiodo-thyronin (T3) or to organophosphate compounds (Chlorpyrifos, Phentoate, belonging to the thionophosphate family of chemicals) at different concentrations.

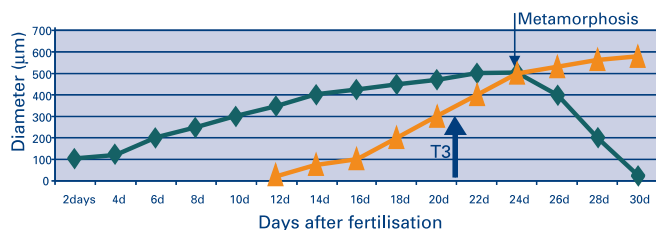


Figure 5: Reciprocal growth of larva (green) and rudiment (orange line). Metamorphosis was enhanced by introduction of 10^{-5} M T3 in rearing sea water.

Results

The exposure since 10 days development to 10^{-6} M thyroid hormone T3 caused dismantling of larval structures at all the stages of urchin rudiment. When used on competent larvae, metamorphosis took place within 5 days, in the absence of environmental stimuli, while control larvae metamorphosed in 6 days more, in the presence of environmental stimuli (e.g. appropriate biofilm), figure 5. Exposure to T3 caused dismantling of the larval structures at all the larval stages (figures 6a, 6b), and when the rudiment was not completely formed, caused release of immature rudiment, without the skeletal structures (spines, teeth) and a number of tube feet from 2 to 5 (figure 6c). The effects of neurotoxic pesticides on the hormone reception were checked by exposing sea urchin embryos, since two days after fertilisation, to two active OP molecules: chlorpyrifos (CPF) and phentoate (Phe) at concentrations ranging around the no observed effect concentration (NOEC) for man (10^{-7} to 10^{-8} M). For controls, 10^{-4} and 10^{-5} M concentrations of the same pesticides were used, that are the doses causing 50% acetylcholinesterase activity inhibition in most models (result of Zoltan Raknoczay, SENSPESTI midterm report, 2004).

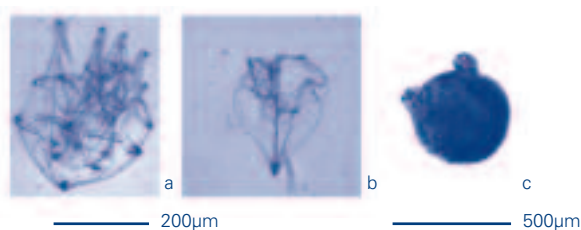


Figure 6: Fifteen days larvae: a: control 8 arm larva; b: larva exposed to 10^{-5} M T3 since 24 h after fertilisation; c: immature rudiment.

At the first endpoint, corresponding to the first week of development, 75% of larvae exposed to the low doses of OPs appeared enhanced as compared to controls, showing the formation of the 3rd pair of perioral arms. After 20 days control and exposed embryos were divided each into two aliquots, and 10^{-6} M thyroid hormone T3 was added in the following way: control, control + T3; exposed to OPs (10^{-7} and 10^{-8} M CPF and Phe, respectively) and exposed to OPs + T3, in total 10 tanks. All the larvae exposed to T3 enhanced metamorphosis, the ones exposed to 10^{-8} OPs+T3 in 3 days, while the control ones + T3 in 5 days. The larvae grown in the presence of 10^{-7} Phe and exposed to T3 in 3 days underwent metamorphosis, releasing on the substrate immature juveniles, with lethal anomalies, as described above. The same anomalous aspect was caused by exposure of larvae to 10^{-4} and 10^{-5} M OPs (without T3 or other stimuli exposure), figure 7.

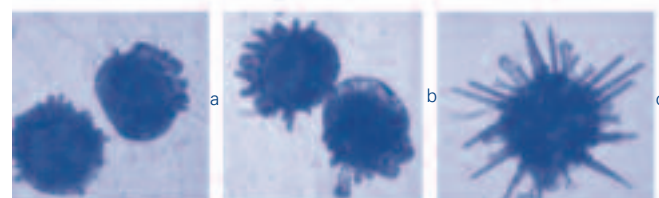
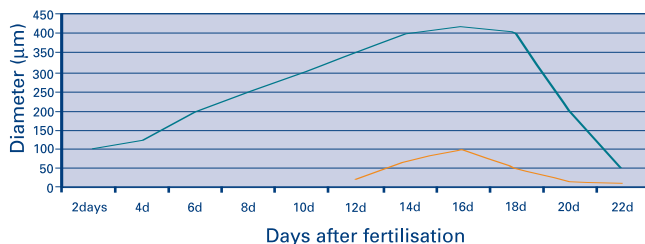


Figure 7: Reciprocal growth of larva (green) and rudiment (orange line). In the presence of 10^{-4} M chlorpyrifos (CPF) or phentoate, the larval structures are reabsorbed, and the immature rudiment is released: a: 10^{-4} M CPF added at 15 days development; b: 10^{-5} M CPF added at 15 days development; c: control.

Discussion

As exposure to 10^{-5} M carbachol (a cholinomimetic agonist of the muscarinic acetylcholine receptors) also caused precocious metamorphosis and consequently the same immature and anomalous juveniles (Falugi and Angelini, 2002), we propose the hypothesis that the effect of OPs is due to inhibition of AChE activity. This allows an increased accumulation of acetylcholine at the receptorial sites, mimicking a thyroid-like response, or interfering with the correct signals that the larva should receive from the juvenile, that in normal conditions when it is fully grown recognizes the suitable substrate and releases hormonal messages. An alternative hypothesis may be proposed, based on the fact that thyroglobulin, the precursor protein of T3, is an AChE (non catalytic) homolog (Grisaru *et al.*, 1999), and that alteration in AChE due to the link of phosphate groups may somehow enhance thyroglobulin iodination.

Maria Grazia Aluigi, Marco Sgro, Mario Franzoni, Cristiano Angelini, Lorenzo Gallus, Carla Falugi

University of Genova, Department of Biology, Lab. of Experimental Embryology and Cytotoxicology, Genova, Italy

Chino Y, Saito M, Yamasu K, Suyemitsu T and Ishihara K (1994). Formation of the adult rudiment of sea urchins is influenced by thyroid hormones. *Dev Biol.* 161: 1-11.

Coniglio L, Morale A, Angelini C and Falugi C (1998). Cholinergic activation of settlement in *Ciona intestinalis* metamorphosing larvae. *J. Exp. Zool.* 280, 314-320.

Faimali M, Falugi C, Gallus L, Piazza V and Tagliaferro G (2003). Involvement of acetylcholine in settlement process of *Balanus amphitrite*. *Biofouling* 19 (Suppl), pp 213-220.

Falugi C and Angelini C (2002). Sea urchin development from the egg to metamorphosis: an integrated model for cell-to-cell and environment interaction. In: Yokota Y, Matranga V, Smolenicka Z (Eds) *The Sea Urchin. From Basic Biology to Aquaculture*. Balkema Pub, Lisse, Abingdon, Exton (PA), Tokyo, pp 73-94.

Grisaru D, Sternfeld M, Eldor M, Glick D and Soreq H (1999). Structural roles of acetylcholinesterase variants in biology and pathology. *Eur. J. Biochem.* 264, 672-686.

Heyland A and Hodin J (2004). Heterochronic developmental shift caused by thyroid hormone in larval sand dollars and its implications for phenotypic plasticity and the evolution of nonfeeding development. *Evolution Int J Org Evolution* 58(3):524-538.

Johnson LG (1998). Stage-dependent thyroxine effects on sea urchin development. *New Zeal. J. Mar. Freshwater Res.* 32: 531-536.

Johnson LG (1997). Thyroxine's evolutionary roots. *Persp. Biol. Med.* 40:529-535.

Johnson LG and Cartwright C.M (1996). Thyroxine-accelerated larval development in the crown-of-thorns starfish, *Acanthaster planci*. *Biol. Bull.* 190:299-301.

Morse DE (1993). Signalling in planktonic larvae. *Nature* 363: 406.

Results of the COMPRENDO project

Final report on project results published

The COMPRENDO project's final publishable report provides an executive summary of the project's findings and is now available on the COMPRENDO website at www.comprendo-project.org

COMPRENDO final workshop presentations

Presentations from the final project workshop which was held in March 2006 in Frankfurt, Germany are also available online at www.comprendo-project.org

Advancing the regulation of mixtures

EDEN workshop on testing strategies and regulatory efforts

Options for incorporating knowledge about endocrine disrupter low-dose and mixture effects into testing strategies and regulatory efforts were discussed at a workshop in Granada, Spain in May 2006. As part of a work package of the EDEN project to advance the regulation of mixtures, experts were drawn from different fields and interests, including regulation, science and NGO's. The results of this workshop will be published as a report and will be publicly available towards the end of 2006.

International conference on food contaminants and neurodevelopmental disorders

December 3 – 5, 2006, Valencia, Spain

This conference to be held in the Museu de les Ciències Príncipe Felipe of Valencia aims to facilitate information exchange and collaboration between researchers participating in different European projects dealing with the neurotoxic and neurodevelopmental effects of food contaminants. A major objective is to discuss the possible implications of the research results on EU policy concerning food contaminants, with the participation of leading representatives from the European Commission research program, the European Food Safety Authority (EFSA), and the expert researchers. More information is available at www.fundacioncac.es/eng/fundacion/actividades/actividadesficha.jsp?idActividad=64

Copenhagen workshop on endocrine disrupters

Consumer products and endocrine disrupters: possible effects on populations

May 30 – June 2, 2007, Copenhagen, Denmark

The 4th Copenhagen Workshop on Endocrine Disrupters to be held at Rigshospitalet will review trends in human reproduction and possible effects of endocrine disrupters on human populations, the effects of endocrine disrupters on gonadal as well as non-gonadal organs, effects of mixtures of endocrine disrupters as well as cellular and genetic aspects of the action of endocrine disrupting chemicals. Further information is available from Dr Anna-Maria Andersson anna@rh.dk

Josephus Gerardus Vos

26 February 1941 – 15 May 2006

After a long illness Professor Josephus Vos passed away on May 15, 2006 at Bilthoven, The Netherlands. He will be greatly missed. Professor Vos was known to many as Jeff and amongst many professional responsibilities coordinated the FIRE project at RIVM (Netherlands National Institute of Public Health). Our sympathy is with his family and collaborators.

Ragnor Pedersen and Andreas Kortenkamp

Participating projects

COMPRENDO

Coordinator: Dr Ulrike Schulte-Oehlmann
www.comprendo-project.org

EDEN

Coordinator: Dr Andreas Kortenkamp
www.edenresearch.info

EURISKED

Coordinator: Prof. Wolfgang Wuttke
www.eurisked.org

FIRE

Coordinator: Prof. Antoon Opperhuizen
www.rivm.nl/fire

Associated projects

ACE	ENDOMET
BONETOX	GENDISRUPT
EASYRING	MENDOS
EDERA	SENSPESTI

See www.credocluster.info

CREDO coordination

For further information and to be kept informed of developments within CREDO please contact:

Dr Andreas Kortenkamp
andreas.kortenkamp@ulsop.ac.uk
Tel/fax: +44 20 7753 5908

Dr Ragnor Pedersen
ragnor.pedersen@ulsop.ac.uk
Tel/fax: +44 20 7753 5811

Centre for Toxicology
The School of Pharmacy, University of London
29-39 Brunswick Square, London WC1N 1AX
United Kingdom

www.credocluster.info

Contact at the European Commission:

Dr Tuomo Karjalainen
Tuomo.Karjalainen@cec.eu.int

Dr Kirsi Haavisto
Kirsi.Haavisto@cec.eu.int

© Dr Andreas Kortenkamp, 2006

Disclaimer

While every effort has been made to provide correct information, we assume no responsibility for the accuracy of the information. Although this describes EU-funded projects, neither the European Union nor the European Commission shall be held responsible for the content, nor the views expressed here.



The CREDO cluster is funded by the European Commission's Fifth Framework Programme for research, technological development and demonstration activities in the European Community.

A joint project funded by two thematic programmes: Quality of Life and Management of Living Resources Programme, and Energy, Environment and Sustainable Development Programme.

