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Review

The European Long-range Research Initiative (LRI): A decade of contributions to human health protection, exposure modelling and environmental integrity

Jacob de Boer[a,*], Ellen Fritsche[b], Greet Schoeters[c], Ian Kimber[d]

[a] VU University, Institute for Environmental Studies, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands
[b] IUF-Leibniz Research Institute for Environmental Medicine, Auf'm Hennekamp 50, 40225 Düsseldorf, Germany
[c] VITO Unit for Environmental Risk and Health, Boeretang 200, 2400 Mol, Belgium
[d] University of Manchester, Faculty of Life Sciences, Manchester M13 9PT, United Kingdom

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ABSTRACT

The European Long-range Research Initiative (LRI) was launched in 2000. The objective of this programme is to provide increased understanding of the potential impact of chemicals on human health and the environment. The aim has been to reduce uncertainty associated with innovation, and to promote evidence-based decision making. In pursuing these objectives the LRI has commissioned independent scientific research in institutions throughout Europe and beyond. The portfolio of research supported by the LRI has delivered significant contributions to risk assessment sciences. In addition, the LRI programme has benefited the broader scientific community. In this review article members of the Cefic European Scientific Advisory Panel (ESAP), the body charged with providing oversight of the LRI programme, illustrate some of those achievements by reference to specific areas of research (respiratory allergy, human biomonitoring, environment and wildlife), and also the contribution made to the development of European scientists through the annual LRI Award Programme.

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1. Introduction

The Long-range Research Initiative (LRI) was launched in the USA in 1996. The objective was then, and remains still, to address important issues and stakeholder concerns in a proactive way by commissioning independent scientific research.

The European LRI, sponsored by Cefic (the European Chemical Industry Council) began a little later in 2000. This programme seeks to increase understanding of the potential impact of chemicals on human health and the environment. The overall purpose of the LRI is to reduce uncertainty associated with innovation, and to strengthen decision-making based on sound scientific evidence. In addressing these objectives the focus is on commissioning independent scientific research that will provide the foundations for effective risk assessment and risk management. Once per year, in June, there is a call for proposals (Fig. 1). The evaluations are carried out by panels composed of managers of the

* Corresponding author.
E-mail address: jacob.de.boer@vu.nl (J. de Boer).

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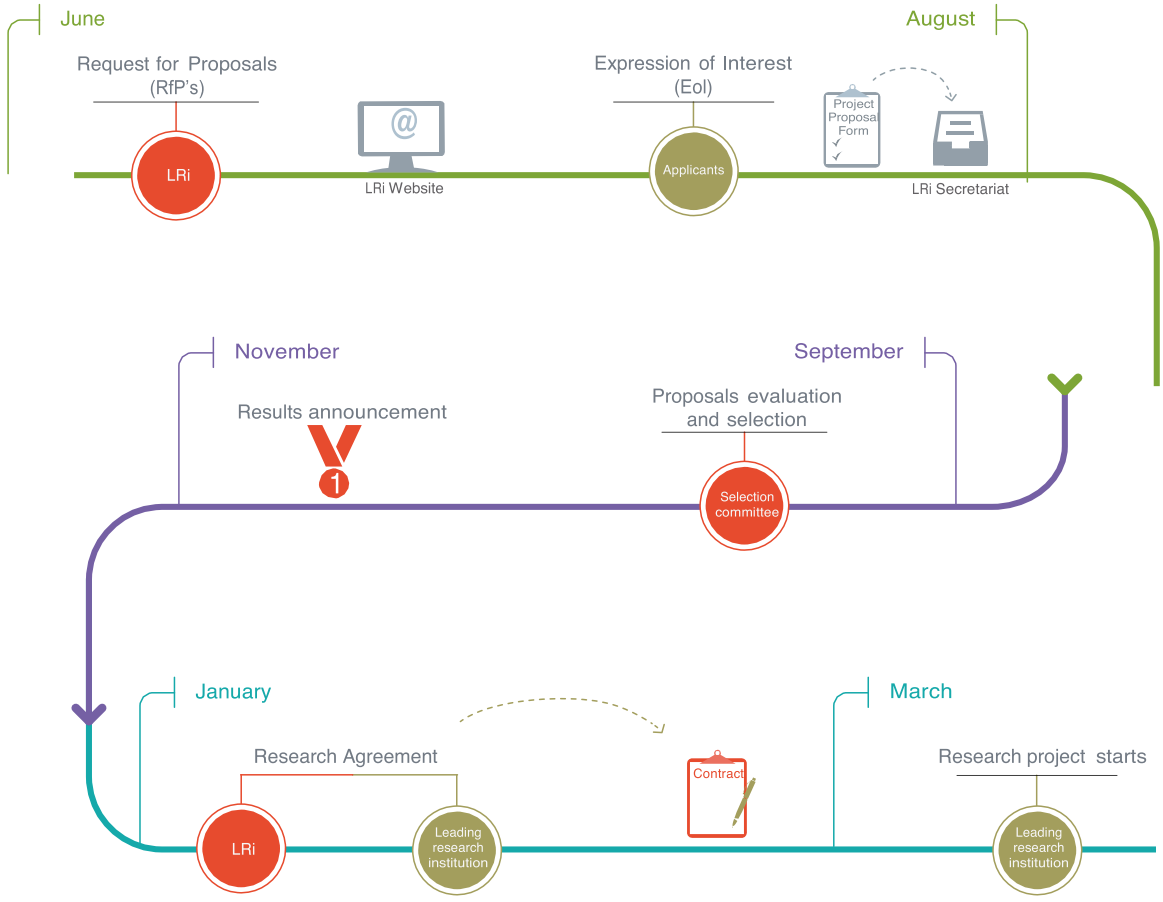

Fig. 1. Annual cycle of LRI project proposals.

chemical industry and scientific experts from independent organisations across Europe. Each year in November, a workshop is being held in Brussels, Belgium at which the project results are being presented and discussed.

In pursuing these aims the LRI has supported research by leading scientists and institutes throughout Europe and beyond. As well as providing the basis for improved risk assessment, the European LRI investment in research has yielded significant dividends to the wider scientific community. The purpose of this short article, authored by members of the Cefic European Scientific Advisory Panel (ESAP), is to highlight, in certain selected areas of research investment, the contribution the European LRI has made to the science of toxicology and environmental safety.

It is important to emphasise that this is not an exhaustive survey of research that has been supported by the European LRI; that programme of work is extensive and details are available elsewhere (Cefic LRI projects, http://www.cefic-lri.org/projects). The purpose is rather to highlight some of the research investments made by the LRI and their relevance to human health and environmental safety.

The selected areas summarised below are: chemical respiratory allergy, human biomonitoring, environmental integrity and wildlife protection and a survey of research in toxicology and environmental impact that has been recognised by the prestigious LRI Innovative Science Award programme. An overview of the projects discussed is given in Table 1.

2. Chemical respiratory allergy

Chemical allergy is an important environmental and occupational health problem, and can take a variety of forms. The most common form of chemical allergy, and the most frequent manifestation of immunotoxicity in humans, is skin sensitisation resulting in allergic contact dermatitis. Many hundreds of chemicals have been shown to cause skin sensitisation and the reactions they cause can sometimes be severe. However, there are tools in place (established *in vivo* tests, new *in vitro* methods and other emerging approaches) that provide the basis for hazard identification, hazard characterisation and risk assessment (Kimber et al., 2011; Basketter et al., 2013).

The picture is somewhat different with another form of chemical allergy that results from sensitisation of the respiratory tract. Chemicals that cause respiratory allergy are far fewer in number, the most commonly implicated being the diisocyanates, acid anhydrides and chloroplatinate salts. Sensitisation of the respiratory tract can result in occupational rhinitis and asthma, and reactions can be fatal (Baur, 2013; Kenyon et al., 2012).

The challenge for toxicologists is that there are currently in place no validated methods of any description for the identification and characterisation of chemicals that have the potential to cause allergic sensitisation of the respiratory tract. There has been for some time a pressing need for approaches that provide this information (Kimber et al., 2014a,b).

Table 1
Overview of LRI projects discussed. A complete overview of all LRI projects can be found at http://cefic-lri.org/projects/.

LRI project no.	Title	Principal investigator, country
	Chemical respiratory allergy	
IA4.6	A rat IGE test for the identification of chemical respiratory allergens	R. Dearman, UK
IRTA2-001-SYNG	Transcript profiling and cytokine fingerprinting: identification and characterisation of chemical respiratory allergens	R. Dearman, UK
IRTA2-001-VITO	*In vivo* and *in vitro* characterisation of chemical respiratory allergens: development of an *in vitro* assay using gene expression profiling	R. van den Heuvel, Belgium
IRTA2-001-HSL	Development of a human *in vitro* testing model to distinguish immune responses to chemical respiratory *vs* contact allergens	G. Evans, UK
R2-TNO	The role of irritancy and irritant-induced inflammation in the elicitation of respiratory hypersensitivity reactions	J. Arts, The Netherlands
IRTA2-002-UUIR	Heterogeneity in response to *iso*-cyanate exposure in the work environment	D. Heederik, The Netherlands
	Human biomonitoring	
D1.1-NOFE	Biomarkers of exposure trends and key developments	M. Jakubowski, Poland
D2.1-MRCI	Background incidence of key biomarkers of chemical exposure within the general population	L.S. Levy, UK
D1.1-SYNG	Study of the role and application of biomarkers in the management of the health risks associated with occupational exposures to chemicals	W. Watson, UK
D2.2-VITO	Generation and application of a model to determine intra- and inter-individual variation in biomarkers	G. Schoeters, Belgium
HBM2-DOW	Development of a tiered set of modelling tools for derivation of biomonitoring guidance values	M. Bartels, USA
HBM3	Data on *in vitro* metabolism and mechanisms of action in combination with kinetic modelling: integrating in risk assessment	B. Blaauboer, The Netherlands
	Environment and wildlife	
EMSG21	Endocrine disruption in the marine environment (EDMAR)	Y. Allen, UK
EMSG22	Endocrine disruption in the aquatic environment; laboratory investigation of endocrine-active chemicals	J. Sumpter, UK
EMSG23	Endocrine modulating effects in fish along the elbe river and in reference areas. assessment of risks related to the habitat conditions and the natural variability of endocrine functions	L. Karbe, Germany
EMSG38	Review of the relative contribution of industrial chemicals, compared to steroids, in causing the sexual disruption in wild fish populations	S. Jobling, UK
EMSG28	Development, validation and application of *in vitro* and *in vivo* test systems for non-oestrogenic endocrine disrupting chemicals in wildlife	A.J. Murk, The Netherlands
EMSG36	Further development and scientific evaluation of appropriate methods for identification and characterisation of toxic effects of hormonally active substances on reproduction in birds as part of multi-generation-studies	I. Chahoud, Germany
EMSG42	Increase of the robustness of the fish endocrine assay and avoidance of irrelevant endpoints	K. Schneider, Germany
EMSG43	Endocrine disrupting effects in fish induced by parasites	W. Kloas, Germany
EMSG27	Environmental effects on uterine tissues of baltic seals with special emphasis on organochlorines and uterine leiomyomas	M. Olsson, Sweden

A number of possible approaches for the predictive assessment of chemical respiratory allergens has been proposed. These have been based on rodent models, or more recently on *in vitro* or *in silico* methods (Boverhof et al., 2008). The European LRI has recognised the importance of encouraging work in this area and has supported a number of research projects that have sought to explore the behaviour of chemical respiratory allergens in various model systems.

One approach that was explored with support from the LRI was a method based upon measurement of induced changes in the serum levels of IgE immunoglobulin in Brown Norway (BN) strain rats (Project IA4.6). Previous work in mice had indicated that changes in the total serum concentration of IgE might provide a biomarker of immune responses provoked by exposure to chemical respiratory allergens. Although initial studies in mice had been encouraging, it was subsequently found that IgE levels in mice were too unstable and too variable to provide a reliable correlate of respiratory sensitising activity. Studies in BN rats revealed that in this species changes in serum IgE levels were insufficiently sensitive to provide a basis for the routine identification of chemical respiratory allergens. Those studies served to confirm that alternative strategies should be sought, and one option was provided by investigations performed in parallel to those summarised above, and which were based on an approach known as cytokine fingerprinting.

Cytokine fingerprinting describes the characterisation of cytokine expression profiles provoked in mice or rats following topical exposure to chemical allergens. It had been shown previously in mice that contact allergens and chemical respiratory allergens provoke different cytokine expression profiles, and that observation raised the possibility that it might be feasible to identify chemicals with the potential to induce sensitisation of the respiratory tract as a function of defined cytokine signatures (Dearman and Kimber, 2001). The LRI supported further examination of cytokine fingerprinting in mice and rats, coupled with an assessment of chemical allergen-induced changes in the expression of other genes that might discriminate between skin and respiratory sensitizers (Project IRTA2-001-SYNG). Those investigations were conducted in parallel in the UK and the Netherlands and confirmed that: (a) cytokine fingerprinting provided a promising approach for the predictive identification of chemical respiratory allergens and (b) that although changes in the expression of certain non-cytokine genes were found to display some bias for contact or respiratory allergens, these markers were neither sufficiently selective, or sufficiently sensitive, to provide a basis for hazard identification.

Since that investment by the European LRI cytokine fingerprinting has continued to be developed, although the complexity of the approach has made widespread application difficult, and the method has not been validated. Other approaches have also been supported (Project IRTA2-001-VITO; Project IRTA2-001-HSL).

Two other aspects of chemical respiratory allergy were explored in research supported by the European LRI.

The first of these was an examination of the interplay between irritation in the airways and the elicitation of chemical respiratory allergy (Project R2-TNO). It had been assumed prior to these

investigations that respiratory irritation would be associated with more aggressive allergic reactions. In fact the opposite was found to be the case, and that prior irritation served to reduce the severity of respiratory allergic reactions induced by inhalation challenge. Those observations caused a re-evaluation of the risk factors that might impact on the severity or duration of allergic reactions in the lungs.

The second area that received support from the European LRI was consideration of selected clinical aspects of chemical respiratory allergy. A cross-sectional survey was conducted among approximately 600 workers using diisocyanates; a common cause of occupational asthma (Project RTA-002-UUIR). In common with previous investigations it was found that respiratory symptoms were commonly not associated with specific IgE antibody. It was concluded that, compared with protein respiratory allergy, respiratory allergy induced by chemicals shows a much weaker alignment between symptoms and IgE antibody or other serological biomarkers. These investigations highlighted and confirmed that chemical respiratory allergy has some unique clinical features.

Investment from the LRI in this area provided an opportunity to explore in greater detail proposed methods for the identification of chemical respiratory allergens – and specifically those based on the elicitation of IgE responses and cytokine fingerprinting. The latter method was found to be a promising approach for hazard identification. Investment in this area also permitted an examination of the relationship between airway irritation and the elicitation of respiratory allergic reactions, and further characterisation of the association of diisocyanate occupational asthma and IgE antibody production.

3. Human biomonitoring

Chemicals are part of our life and our environment and consequently they enter the human body by ingestion, in halation or dermal exposure. The presence of chemicals in the environment and in food is well monitored, but only fragmented information is available on the levels in humans. Human biomonitoring measures directly the levels of chemicals in blood, urine or other human tissues or fluids (Angerer et al., 2007). It is important to keep track of the concentrations in humans to ensure that they do not pose hazards and are kept at levels without adverse health effects even for the most sensitive individuals in the population such as babies, elderly or sick people. The LRI program contributed and provided knowledge on monitoring chemical exposure in the general population, how to optimise biomonitoring programs and how to interpret and use this information.

The first projects (D1.1-NOFE, D2.1-MRCI, D1.1-SYNG) reviewed the potential of human biomonitoring. Similar and perhaps even more stringent than environmental sampling and monitoring, human biomonitoring requires sampling and analytical quality assurance and biomarker validation. The increased sensitivity and performance of analytical techniques in recent years have allowed to develop new biomarkers that are sensitive enough to trace chemicals at very low levels in the general population. Reactive chemicals may form adducts with macromolecules in the body, while urinary metabolites often reflect acute exposures. Integration of the biomarker information with genomic and proteomic data should lead to identification of individual susceptibility and better prediction of risks and prevention of diseases related to chemical exposures (Watson and Mutti, 2004).

More than 300 biomarkers are regularly measured in the human biomonitoring program connected to the US National Health and Nutrition Examination Survey (NHANES) which includes individuals between 6 and 60 years (Calafat, 2012). Two LRI projects studied background incidences of a range of biomarkers in Europe. Project D2.1-MRCI conducted a cost effective postal based survey in the UK and provided reference ranges of urinary biomarkers for benzene, chlorinated hydrocarbons, dithiocarbamates, cadmium, mercury, naphthalene, diethylhexyl phthalate, synthetic pyrethroids and xylene in adults (Bevan et al., 2013). The reference values for most biomarkers were similar to the reference values obtained in the US-NHANES program. LRI project D.2.2-VITO provided greater insight into inter-individual variability based on human biomarker data from large environmental health programs that have been conducted in recent years in Belgium and in Denmark. The inter-individual variability of marker PCBs, that were analysed in serum lipids of adolescents, was explained for 36% by biological factors, 14% by local factors and 1% by food consumption. PCBs have a long biological half- life of several years. For the short lived (18–20 h) urinary 1-hydroxypyrene which is a biomarker for exposure to polyaromatic hydrocarbons, only 2.5% of the variability between adolescents was explained by biological factors and 2.3% by life style factors. For adults, respective data were 8.5% and 15.7% (Den Hond et al., 2009; Govarts et al., 2010).

Currently, chemicals that are persistent in the body are manufactured only in limited amounts. Most modern compounds are non-persistent and biomarker levels may fluctuate shortly after exposure, certainly if the exposure pattern is discontinuous. The implication is that single spot samples will not only capture the variability of internal dose in the population but also variability in the individual, without disentangling the inter- and intra-individual variability. This may lead to misclassification of individuals with high/low exposures. In LRI project HBM4-VITO a study with 8 volunteers established this intra-individual variability for 4 metals and 15 organic compounds including 9 parabens, triclosan, benzophenon, bisphenol A and yielded information on the determinants of the variability. This was used as input for the development of an easy to use software model [HBM-simulator] that is publically available, free to download and assists with interpretation of studies utilising single human biomonitoring samples for exposure analysis.

Due to increased analytical sensitivity, biomonitoring studies can detect and quantify numerous new chemicals or their metabolites, some of them with little information on toxicology or exposure–response relationships. Our ability to detect hazardous substances (or their metabolites and health effects) often exceeds our understanding of their biological relevance. For data-rich substances biological monitoring guidance values have been derived to allow interpretation of biomonitoring data in terms of increased exposure and/or increased health risk. This mostly applies to biomarkers used in the occupational field. The LRI project HBM1-UCR designed a framework for interpretation of biomarker data in environmental surveillance studies. It comprised four levels defined by increasing data context and hence increasing confidence for human health risk assessment. The utility of the framework was tested by using available data from 12 chemicals (Bevan et al., 2012) and was thereafter refined through a workshop and by consultation with an advisory group. The concept may assist scientists, regulators, and stakeholders in providing guidance for the interpretation of human biomonitoring data (for both groups and individuals), appreciating limitations, and allowing appropriate action to be taken when required.

Relating biomarker levels to external chemical concentrations will further facilitate their interpretation and bridge internal and external doses. Biomarker levels can be linked to external chemical exposures if information is available on uptake routes and kinetics. LRI project HBM2-DOW provided improved reconstruction of external chemical exposure by so called reverse dosimetry and by integration of physiologically based pharmacokinetic modelling with Bayesian inference, and Markov chain Monte Carlo

simulation. The approach allowed derivation of a population estimate of inhalation exposure to *m*-xylene based on exhaled breath and venous blood *m*-xylene and urinary 3-methylhippuric acid measurements in a controlled volunteer study (McNally et al., 2012). The approach has now been taken further and a generic software model [IndusChemFate] is now available as a spreadsheet application in MS Excel for forward dosimetry. Biomarker concentrations in blood or urine can be predicted from external health based acceptable daily doses or acceptable health based daily exposures taking into account the variability in the population (Jongeneelen and Berge, 2011).

If no health risk related guidance values exist for a given chemical, biomonitoring data may be integrated in new concepts of risk assessment. The LRI supported project HBM3 study integrated different areas of knowledge on the chemistry and the biological activity of compounds in combination with *in silico* prediction of metabolites and target tissues, *in vitro* metabolism, kinetics and toxicity studies. The concept has been tested for paracetamol, bisphenol A and 2-butoxyethanol and showed good predictivity of toxicity particularly if metabolites are considered (which remains a limitation of some *in vitro* assays). However, quantitative predictivity remains challenging due to uncertainties and choices of point of departures (Blaauboer et al., 2012).

The LRI projects have timely contributed in exploring the individual variability in exposure to chemicals, which is observed in human biomonitoring studies of the general population. This knowledge facilitates the interpretation of human biomonitoring data and an interpretation framework has been derived. Personal characteristics, environment and life style conditions as well as sampling frames are important influential parameters. The LRI projects have taken up this knowledge and in combination with toxicokinetic modelling ICT based tools have been developed that are publically available and that allow to predict internal human exposure to chemicals. Whether these modelling tools are effective for predicting human exposures to newly emerging chemicals is a challenge for further investigations.

4. Environment and wildlife

Nine LRI projects were funded by Cefic in the area Environment and Wildlife. Eight of these were studies on endocrine disruption including some reproduction studies. This reflects the weight of the discussions on endocrine disruption that took place during the last two decades after Colborn launched the idea of endocrine disruption in 1993 (Colborn, 1993). The causes of endocrine disruption are still a matter of debate. The major discussion is focusing on the question to which extent endocrine disruption might be derived from natural sources, such as steroids and phytoestrogens, or from xenobiotic chemicals, that enter the aquatic environment. The projects under the LRI Environment and Wildlife theme provided substantial knowledge that was useful in this ongoing debate.

In one of the papers coming out of the EDMAR project (EMSG21), Matthiessen (2000) concluded that there was a clear need for more field and semi-field studies explicitly designed to uncover links between pollution effects on individuals, and ultimate impacts on populations and communities, although, as he concluded, this is also true for other relevant chemical pollutants. The EDMAR project investigated whether there was evidence of changes in the reproductive health of marine life and possible causes and potential impacts thereof (Matthiessen et al., 2002). The project included the development of methods for the detection of endocrine disruption in the marine environment (including biomarkers), toxicity identification, studies on biological responses in estuaries and identification of substances and sources causing the effects observed. This project was very productive in terms of scientific papers. Although due to dilution effects of marine sewage discharges in estuaries and coastal waters, fewer effects were expected on marine organisms, research in UK estuaries showed that vitellogenine induction, and to a lesser extent ovotestis and abnormal spermatogenesis, were widespread in flounder (*Platichthys flesus*) (Kirby et al., 2004). Several androgens and estrogens were found to be present in large estuaries, mainly in sediments, but only estrogens appeared to be of major biological significance. Males of at least four estuarine fish species show some feminization, but the implications for fish populations remain unclear: some reduction in reproductive success can probably occur without population-level effects.

Two LRI projects (EMSG22 and -23) focused on endocrine disruption in the aquatic environment. Laboratory studies were conducted in which fish were exposed to defined concentrations of known chemicals. Natural steroids, synthetic steroids and xenoestrogens were assessed. Hecker et al. (2002) found that the endocrine system in wild bream is disrupted in stretches of the Elbe River but that there was no single chemical that alone could explain the observed inhibitory effects on sexual development.

In project EMSG38, Jobling et al. (2006) used roach (*Rutilus rutilus*) to study the influence of steroidal estrogens originating from human excretion in comparison with industrial chemicals in rivers contaminated by treated sewage effluents. The results provided substantive evidence to support the hypothesis that steroidal estrogens play a major role in causing intersex in freshwater fish in UK rivers. The authors showed that the location and severity of these endocrine-disrupting effects can be predicted. No correlation was found between any of the end points measured in the roach and the proportion of industrial effluents entering the rivers.

Project EMSG28 provided information on *in vitro* (T-screen & Keratonisation Assay) and *in vivo* assays (metamorphosis, migration and prolonged FETAX-assay) for alterations of thyroid and retinoid dependent processes. It was meant to bridge the gap between the *in vitro* and *in vivo* assays, and the effects seen in the field on the population level. Challenge experiments (choosing relevant stresses for the endocrine effects observed) with realistically exposed amphibians were conducted. This project was also part of the Dutch National Investigation into Oestrogenic Compounds (LOES) program (Schriks et al., 2002).

Project EMSG36 resulted in six scientific papers. As an alternative approach to the design of existing reproduction studies, new endpoints facilitating the detection of hormone-mediated effects were studied. In particular, questions raised by the OECD (Avian Two-Generation Toxicity Test in the Japanese Quail. First Draft December 1999) were addressed. Experiments were performed on the Japanese quail (*Coturnix coturnix japonica*) for which an extensive historical database is available. The regular determination of spermatid count, serum concentrations of testosterone (both sexes), estradiol (in females only) and sex ratio were recommended because those variables provided promising measurement endpoints (Selzsam et al., 2005). Grote et al. (2008) tested the triazole fungicide Epox and concluded that dietary treatment of Japanese quail with 50 and 500 ppm of Epox resulted in a clear impact on the testis. The evaluation of the additional endpoints spermatid count and testicular histology were recommended for future studies on avian reproduction.

The objective of project EMSG42 was to determine the influence of the test compounds potassium permanganate, 2-methoxyethanol and octanol to reproduction and the endocrine system of fathead minnow (*Pimephales promelas*). For this purpose, mature male and female fathead minnow were exposed in a flow-through test system to aqueous test media containing the test item at various concentrations under defined conditions. It appeared that male fathead minnows guard the spawning ground and are

strongly territorial during the spawning period. That behaviour stresses the animals on top of possible stress caused by a chemical. A high mortality was observed. Therefore, the feasibility of the testing design should be better with non-territorial species.

One natural aspect rarely considered in ecotoxicological studies is how parasites modulate host physiology. In project EMSG43 investigations were carried out to characterize the impacts of the parasitic tapeworm *Ligula intestinalis* on plasma sex steroid levels and expression of key genes associated with the reproduction in roach (*Rutilus rutilus*). Parasitisation by *L. intestinalis* suppressed gonadal development in both genders of roach and analysis of plasma sex steroids revealed substantially lower levels of 17β-oestradiol (E2) and 11-ketotestosterone (11-KT) in infected females as well as E2, 11-KT, and testosterone in infected males. Consistently, in both infected females and males, expression of the estrogen dependent genes such as vitellogenin and brain-type aromatase in liver and brain was reduced. The authors concluded that parasites like *L. intestinalis* can induce endocrine disruption-like effects in fish and modulate classical biomarkers of endocrine disruption (Trubiroha et al., 2010).

The only project not addressing endocrine disruption under the Environment and Wildlife theme was project EMSG27, which investigated whether leiomyomas (non-cancerous growths of the uterus) in grey seals, collected during the period of decreased levels of organochlorines were in a regressive phase and whether the period of increased levels of organochlorines caused the leiomyomas to proliferate. In addition, *in vitro* studies were conducted to characterise the growth control mechanism of grey seals leiomyoma cells. Backlin et al. (2003) concluded that it is possible that the development of leiomyomas in seals is associated with organochlorines and the previously low reproductive activity.

In summary, investment from the LRI in environment and wildlife studies has predominantly been on the subject of endocrine disruption. Although the eight projects on this topic have delivered a substantial amount of information and a series of scientific papers, they could not conclude the debate on endocrine disruption. Still, questions remain on the endocrine activity of xenobiotic chemicals at the level at which they occur in the environment and in organisms. Meanwhile, other emerging chemicals have been found in the environment and studies on their possible effects, whether or not in connection with other chemicals are also needed.

5. Ten years of the Cefic—LRI award

In 2004, the annual LRI Innovative Science Award was launched, and since then this has been supporting outstanding, innovative research ideas with a total sum of € 1,000,000. With this prestigious award young European-based scientists have been given the opportunity to realize their ideas and thus enhance science to help protect human health and the environment. By this means, the Cefic—LRI award program created a platform to not only promote short-term research projects, but also induce sustainability within the funded research areas as individual history of the award winners show persistence of their subjects in the respective fields.

The first award in 2004 was given to Roger Godschalk, Maastricht University, NL for a proposal on genetic risks (Verhofstad et al., 2008). Between 2004 and 2013, he has been publishing nineteen articles on this topic indicating the long-standing effect of the LRI award on the area of research of the awardee. Roger Godschalk developed new approaches to test germ line mutagenicity in humans exposed to environmental and dietary mutagens, investigated the role of polymorphisms in xenobiotic metabolising enzymes and dietary intervention for generation of possible carcinogens and developed biomarkers of carcinogen exposure. He currently holds a position as Assistant Professor of Toxicology working on Genetic Toxicology and Molecular Epidemiology at Maastricht University, The Netherlands.

In 2005, Paul van den Brink (Van den Brink and Kater, 2006) from Wageningen University, NL received the second LRI award for proposing ecological risk assessment of chemicals. Since then, he has been publishing more than sixty papers on this topic. Extracts of his extensive work are exemplified here by investigations on exposure of wildlife and sediments in different countries comparisons of field studies to laboratory toxicity data, investigations on species differences in response to environmental chemicals and development of new concepts for ecological risk assessment. He is Professor for Chemical Stress Ecology at Wageningen University, NL performing ecological risk assessment of chemicals and honorary visiting Professor at the University of York, UK.

The first female receiving the LRI award was Ellen Fritsche, Düsseldorf, Germany, for scientific development of a human 3D neurosphere *in vitro* model for developmental neurotoxicity (DNT) testing in 2006 (Moors et al., 2009). She has characterized this model, expanded it to rodents for investigating species differences within signalling pathways, adopted it to medium throughput for application in a testing context and participated in approaches to use alternative methods for risk assessment. As a Professor for Environmental Toxicology she heads the research group for Sphere Models and Risk Assessment at the IUF—Leibniz Research Institute for Environmental Medicine in Düsseldorf, Germany.

Roman Ashauer from the Swiss Federal Institute of Aquatic Science and Technology, Switzerland, was honoured by the LRI award for a proposal on aquatic ecotoxicology in 2007. He proposed development and usage of toxicokinetic and toxicodynamic models including organism recovery for improved ecotoxicological risk assessment, especially under dynamic exposure. Since then he has been publishing a large number of papers on different models for ecological hazard and risk assessment, including species differences in responses to pesticides and also mechanisms of aquatic ecotoxicity (Nyman et al., 2013). He is currently Anniversary Research Lecturer at the University of York, UK.

In 2008, Emma Taylor from the MRC Toxicology Unit, Leicester University, UK won the LRI award for her research proposal on the investigations of male epigenetic transgenerational chemical toxicity using *in vivo* and *in vitro* stem cell based systems. Thereby, she discovered the emerging fundamental roles for non-coding RNA species in toxicology and developed novel genomic methods for drug discovery and mechanism-based toxicological assessment (Gant et al., 2009). She is now still actively working in toxicological research at the Centre for Radiation, Chemical and Environmental Hazards in Oxford, UK.

The LRI award in 2009 was given to Hector Keun from the Imperial College, London, UK, who proposed the usage of metabonomic biomarkers to bridge the gap between environmental exposure and human disease. Since then he has been publishing more than thirty papers on the issue of metabolome analyses covering nuclear magnetic resonance (NMR)-based metabolic profiling and biomarker assessment (Beckonert et al., 2010) in *e.g.* tissue regeneration, development, cancer, and after exposure towards toxins. The broad application domain for this technique, which can even be applied in intact tissues (Beckonert et al., 2010), demonstrates its potential power for future application in toxicological risk assessment. Hector Keun currently holds a position as a senior Lecturer at the Imperial College and expands the use of metabolomics to drug discovery and development.

In 2010 the LRI award went to Juana Maria Delgado-Saborit from Birmingham University, UK. Her research proposal 'In quest of

new fingerprints of exposure to volatile organic compounds (VOC) from consumer products' aimed at bringing more insight to the potential impacts of VOCs emitted from consumer products in indoor environments on health at the low levels of exposures. She has been assessing human exposure towards VOC (Delgado-Saborit et al., 2011), polycyclic aromatic hydrocarbon (PAH) mixtures, combustion-related pollutants as well as nanomaterials thus contributing to risk assessment on the exposure side. Juana Maria Delgado-Saborit currently holds a position as a Lecturer in Environmental Health at the University of Birmingham, UK.

Thomas G. Preuss from the RWTH Aachen, D, was awarded with the LRI award in 2011. His research proposal 'Improving mechanistic understanding of population recovery for aquatic macroinvertebrates' aimed to develop a scientifically-based approach for the prediction and measurement of recovery for environmental risk assessment. He has been contributing to the field of ecotoxicology since by investigating the influence of reproduction on population vulnerability, identifying vulnerable species for ecotoxicological risk assessment in the EU, defining life-stage-dependent sensitivity of aquatic organisms to organic pollutants and studying the combined effects of chemical and natural stressors on populations (Gergs et al., 2013). Currently, Thomas Preuss is the leader of the group 'mechanistical stress ecology' at the RWTH Aachen, Germany.

The LRI award in 2012 was given to Andreas Bender, University of Cambridge, UK. Andreas Bender is a Lecturer for Molecular Informatics with the Unilever Centre for Molecular Science Informatics at the University of Cambridge. Until April 2010 he was an Assistant Professor for Cheminformatics and Pharmaceutical IT with the Leiden/Amsterdam Center for Drug Research and Head of the Pharma-IT Platform at Leiden University, NL. In his work, he is involved with the integration and analysis of chemical and biological data, aimed at understanding phenotypic compound action (such as cellular readouts, and also organism-level effects) on a mechanistic level, ranging from compound efficacy to toxicity (Nguyen et al., 2013).

The 10th LRI award completing the total sum of € 1,000,000 was finally given to Sabine A.S. Langie, Flemish Institute for Technological Research (VITO), B, in 2013. She is interested in studying early-life exposures to xenobiotic agents and/or specific dietary compounds, and how these can influence the interaction between DNA methylation and oxidative stress/DNA damage and predispose to pathological diseases later in life (Langie et al., 2013). Thus, she received the award for her LRI proposal: 'Environmental programming of respiratory allergy in childhood: the applicability of saliva to study the effect of environmental exposures on DNA methylation'.

Looking back on these 10 years of Cefic—LRI award sponsoring, it is impressive how this award promoted awardees building their careers: 100% are still actively working in science, holding faculty positions as Group leaders, Lecturers or Professors. Thereby, the male–female ratio is 60:40 showing almost equal gender distribution. However, not only the personal promotion of this award is stunning, also endorsement of sustainability of the scientific subjects associated with the respective awards is striking: every single awarded topic of the last 10 years has been continued by the awardee. Therefore, the Cefic—LRI award is an outstanding tool for supporting research innovation in a long-lasting fashion.

References

Angerer, J., Ewers, U., Wilhelm, M., 2007. Human biomonitoring: state of the art. Int. J. Hyg. Environ. Health 210, 201–208.

Backlin, B.-M., Eriksson, L., Olsson, M., 2003. Histology of uterine leiomyoma and occurrence in relation to reproductive activity in the Baltic grey seal (*Halichoerus grypus*). Vet. Pathol. 40, 175–180.

Basketter, D.A., Alepee, N., Casati, S., Crozier, J., Eigler, D., Griem, P., Hubesch, B., de Knecht, J., Landsiedel, R., Louekari, K., Manou, I., Maxwell, G., Netzeva, T., Petry, T., Rossi, L.H., 2013. Skin sensitization—moving forward with non-animal testing strategies for regulatory purposes in the EU. Regul. Toxicol. Pharmacol. 67, 531–535.

Baur, X., 2013. A compendium of causative agents of occupational asthma. J. Occup. Med. Toxicol. 8, 1–8.

Beckonert, O., Coen, M., Keun, H.C., Wang, Y., Ebbels, T.M., Holmes, E., Lindon, J.C., Nicholson, J.K., 2010. High-resolution magic-angle-spinning NMR spectroscopy for metabolic profiling of intact tissues. Nat. Protoc. 5, 1019–1032.

Bevan, R., Angerer, J., Cocker, J., Jones, K., Koch, H.M., Sepai, O., Schoeters, G., Smolders, R., Levy, L., 2012. Framework for the development and application of environmental biological monitoring guidance values. Regul. Toxicol. Pharmacol. 63, 453–460.

Bevan, R., Jones, K., Cocker, J., Assem, F.L., Levy, L.S., 2013. Reference ranges for key biomarkers of chemical exposure within the UK population. Int. J. Hyg. Environ. Health 216, 170–174.

Blaauboer, B.J., Boekelheide, K., Clewell, H.J., Daneshian, M., Dingemans, M.M., Goldberg, A.M., Heneweer, M., Jaworska, J., Kramer, N.I., Leist, M., Seibert, H., Testai, E., Vendebriel, R.J., Yager, J.D., Zurlo, J., 2012. The use of biomarkers of toxicity for integrating in vitro hazard estimates into risk assessment for humans. ALTEX 29, 411–425.

Boverhof, D.R., Billington, R., Gollapudi, B.B., Hotchkiss, J.R., Krieger, S.M., Pole, A., Wiecinski, C.M., Woolhiser, M.R., 2008. Respiratory sensitization and allergy: current research approaches and tools. Toxicol. Appl. Pharmacol. 226, 1–13.

Calafat, A.M., 2012. The US National Health and Nutrition Examination Survey and human exposure to environmental chemicals. Int. J. Hyg. Environ. Health 215, 99–101.

Colborn, T., 1993. Developmental effects of endrocrine-disrupting chemicals in wildlife and humans. Environ. Health Perspect. 101, 378–384.

Dearman, R.J., Kimber, I., 2001. Cytokine fingerprinting and hazard assessment of chemical respiratory allergy. J. Appl. Toxicol. 21, 153–163.

Delgado-Saborit, J.M., Aquilina, N.J., Meddings, C., Baker, S., Harrison, R.M., 2011. Relationship of personal exposure to volatile organic compounds to home, work and fixed site outdoor concentrations. Sci. Total Environ. 409, 478–488.

Den Hond, E., Govarts, E., Bruckers, L., Schoeters, G., 2009. Determinants of polychlorinated aromatic hydrocarbons in serum in three age classes—methodological implications for biomonitoring. Environ. Res. 109, 495–502.

Gant, T.W., Zhang, S.D., Taylor, E.L., 2009. Novel genomic methods for drug discovery and mechanism-based toxicological assessment. Curr. Opin. Drug Discov. Dev. 12, 72–80.

Gergs, A., Zenker, A., Grimm, V., Preuss, T.G., 2013. Chemical and natural stressors combined: from cryptic effects to population extinction. Sci. Rep. 3, 2036.

Govarts, E., Den Hond, E., Schoeters, G., Bruckers, L., 2010. Determination of serum PCBs in adolescents and adults: regression tree analysis and linear regression analysis. Hum. Ecol. Risk Assess. 16, 1115–1132.

Grote, K., Niemann, K., Selzsam, B., Haider, W., Gericke, C., Herzler, M., Chahoud, I., 2008. Epoxiconazole causes changes in testicular toxicology and sperm production in the Japanese quail (*Coturnix coturnix japonica*). Environ. Toxicol. Chem. 27, 2368–2374.

Hecker, M., Tyler, C.R., Hoffman, M., Maddix, S., Karbe, L., 2002. Plasma biomarkers in fish provide evidence for endocrine modulation in the Elbe river, Germany. Environ. Sci. Technol. 36, 2311–2321.

Jobling, S., Williams, R., Johnson, A., Taylor, A., Gross-Sorokin, M., Nolan, M., Tyler, C. R., van Aerle, C., Santos, E., Brighty, G., 2006. Predicted exposures to steroid estrogens in UK rivers correlate with widespread sexual disruption in wild fish populations. Environ. Health Perspect. 114 (Suppl. 1), 32–39.

Jongeneelen, F.J., Berge, W.F., 2011. A generic cross-chemical predictive PBTK model with multiple entry routes running as application in MS Excel; design of the model and comparison of predictions with experimental results. Ann. Occup. Hyg. 55, 841–864.

Kenyon, N.J., Morrissey, B.M., Schivo, M., Albertson, T.E., 2012. Occupational asthma. Clin. Rev. Allergy Immunol. 43, 3–13.

Kimber, I., Basketter, D.A., Gerberick, G.F., Ryan, C.A., Dearman, R.J., 2011. Chemical allergy: translating biology into hazard characterization. Toxicol. Sci. 120 (Suppl. 1), S238–S268.

Kimber, I., Dearman, R.J., Basketter, D.A., 2014a. Diisocyanates, occupational asthma and IgE antibody: implications for hazard characterization. J. Appl. Toxicol. 34, 1073–1077.

Kimber, I., Dearman, R.J., Basketter, D.A., Boverhof, D.R., 2014b. Chemical respiratory allergy: reverse engineering an adverse outcome pathway. Toxicology 318, 32–39.

Kirby, M.F., Allen, Y.T., Dyer, R.A., Feist, S.W., Katsiadaki, I., Matthiessen, P., Scott, A.P., Smith, A., Stentiford, G., Thain, J., Thomas, K.V., Tolhurst, L., Waldock, M.J., 2004. Surveys of plasma vitellogenin and intersex in male flounder (*Platichthys flesus*) as measures of endocrine disruption by estrogenic contamination in United Kingdom estuaries. Temporal trends 1996–2001. Environ. Toxicol. Chem. 23, 748–758.

Langie, S.A., Achterfeldt, S., Gorniak, J.P., Halley-Hogg, K.J., Oxley, D., van Schooten, F. J., Godschalk, R.W., McKay, J.A., Mathers, J.C., 2013. Maternal folate depletion and high-fat feeding from weaning affects DNA methylation and DNA repair in brain of adult offspring. FASEB J. 27, 3323–3334.

Matthiessen, P., 2000. Is endocrine disruption significant ecological issue? Ecotoxicology 9, 21–24.

Matthiessen, P., Allen, Y., Bamber, S., Craft, J., Hurst, M., Hutchinson, T., Feist, S., Katsiadaki, I., Kirby, M., Robinson, C., Scott, S., Thain, J., Thomas, K., 2002. Mar. Environ. Res. 54, 645–649.

McNally, K., Cotton, R., Cocker, J., Jones, K., Bartels, M., Rick, D., Price, P., Loizou, G., 2012. Reconstruction of exposure to *m*-xylene from human biomonitoring data using PBPK modelling, Bayesian interference and Markov Chain Monte Carlo simulation. J. Toxicol. doi:http://dx.doi.org/10.1155/2012/760281.

Moors, M., Rockel, T.D., Abel, J., Cline, J.E., Gassmann, K., Schreiber, T., Schuwald, J., Weinmann, N., Fritsche, E., 2009. Human neurospheres as three-dimensional cellular systems for developmental neurotoxicity testing. Environ. Health Perspect. 117, 1131–1138.

Nyman, A.M., Hintermeister, A., Schirmer, K., Ashauer, R., 2013. The insecticide imidacloprid causes mortality of the freshwater amphipod *Gammarus pulex* by interfering with feeding behavior. PLoS One 8, e62472.

Nguyen, H.P., Koutsoukas, A., Mohd Fauzi, F., Drakakis, G., Maciejewski, M., Glen, R. C., Bender, A., 2013. Diversity selection of compounds based on 'protein affinity fingerprints' improves sampling of bioactive chemical space. Chem. Biol. Drug Des. 82, 252–266.

Schriks, M., Gutleb, A.C., Van den Berg, J.H.J., Murk, A.J., 2002. Thyroidogenic potency of compounds determined with the T-screen assay. SETAC, Vienna, Abstract no. 18–19.

Selzsam, B., Niemann, L., Gericke, C., Chahoud, I., 2005. Suitability of some additional parameters in reproduction studies in Japanese quail: preliminary experience. Avian Poult. Rev. 16, 41–46.

Trubiroha, A., Kroupova, H., Wuertz, S., Frank, S.N., Sures, B., Kloas, W., 2010. Naturally-induced endocrine disruption by the parasite *Ligula inteestinalis* (Cestoda) in roach (*Rutilus rutilus*). Gen. Comp. Endocrinol. 166, 234–240.

Van den Brink, P.J., Kater, B.J., 2006. Chemical and biological evaluation of sediments from the Wadden Sea, The Netherlands. Ecotoxicology 15, 451–460.

Verhofstad, N., Linschooten, J.O., van Benthem, J., Dubrova, Y.E., van Steeg, H., van Schooten, F.J., Godschalk, R.W., 2008. New methods for assessing male germ line mutations in humans and genetic risks in their offspring. Mutagenesis 23, 241–247.

Watson, W.P., Mutti, A., 2004. Role of biomarkers in monitoring exposures to chemicals: present position, future prospects. Biomarkers 9, 211–242.