REPORT

Conference On
Endocrine Disrupting Chemicals

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At India Habitat Centre

New Delhi

By

Toxics Link
for a toxics-free world

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Background

The research studies claim that manmade chemicals have the potential impact on the endocrine system and may influence with the development process of humans and wildlife species. These chemicals are mostly termed as the Endocrine Disrupting Chemicals (EDCs). EDCs have been suspected to be associated with the altered reproductive function in males and females; increased incidence of breast cancer, abnormal growth patterns and neurodevelopment delays in children, as well as changes in immune function.

In 2002 the International Programme on Chemical Safety (IPCS) developed a report in 2002 entitled " **Global Assessment of the State of the Science of Endocrine Disruptors"** and again in 2012 another report entitled " **State of the Science of Endocrine Disrupting Chemicals 2012**" was published jointly by WHO and UNEP. As per the report of NYU School of Medi cines the cost to the European Union from EDCs is 157 bil l i on EUROs each year The issue of EDCs has been accepted as a global issue by the Strategic A p p r o a c h to International Chemical Management (SAICM), a UN sponsored body to look into the chemical management and countries are in the course to take suitable actions to mitigate the challenges of EDCs.

There are number of chemicals and pesticides those have been linked up with this issue. Globally efforts are being initiated to look into the issue of EDCs critically and to phase out these chemicals from various products. These chemicals are extensively used in manufacture and production of a wide range of products and have the potential of adverse health impacts though much is not known about these chemicals. The Hazardous waste rules in India have stated that the endocrine disruptor is one of the characteristics for the classification of hazardous waste.

Inaugural Session

However, in India as well as in the South Asian region the issue of EDCs has not been in public domain and there is very little information and data on these chemicals though in India few researchers are working on this issue. There is a need to bring this issue into public conversation and engage with all stakeholders to get a better understanding on various dimensions and impacts on use of such chemicals which can only be possible through generation of new information and data also bringing together global knowledge that can be put to use in the region.

Mr. Ravi Agarwal, Director, Toxics Link, welcome and thanked to all the participants of the workshop for joining this deliberation on the emerging public and environmental health issue of EDCs. He briefed the group on similar campaigns taken up by the organisation in the last two decades, such as on dioxins, medical waste and waste to energy plants which helped register toxicity as an issue in both public and policy awareness. He noted the success of these

campaignsciting the example of the Medical Waste Rules in India in 1998 which was a result of medical community becoming joint petitioners in the plea to the Supreme Court. Much work is also ongoing to regulate the waste to energy plants, which release dioxins that work on an endocrine level. He emphasised that EDCs are an urgent matter of public policy, as not only are they all around us in be it plastics or pesticidesbut also because their impact is long term.

Dr. Alok Dhawan, Director, Indian Institute of Toxicological Research, Lucknow, began his address

by briefly outlining the history and nature of work undertaken at his institution on toxics including industrial toxicology, mechanistic toxicology and nano materials. He noted the paucity of enough primary data in India on EDCs and the difficulties in pooling this data given the vastness of the country. But, he added, a lot of work on this issue is underway across the world such as on reproductive toxicity and cancer, which result in prolonged battles between the regulator and industry. He underlined that alongside drafting policies and regulation based on scientific facts, focus should extend to implementation. At the same time, efforts should be made to make people aware and educated. They should understand the risks and benefits of all the products they are using. He concluded that as humans we are the most destructive species and it is our responsibility to leave a healthy environment for the next generation.

Dr. K. S. Sachdeva, Ministry of Health and Family Welfare, submitted that there are little scientific evidence on how EDCs and other pollutants affect health and environment which acts as constraint

for regulatory bodies, but he added that lack of evidence must not prevent us from taking some action and that he came to the meeting precisely to know the existing state of research in India, which the advocates can help standardise for regulatory feeds. He also underscored that as a health administratorhe is aware how governments in developing countries always fall short of resources to monitor the regulations in place, and expressed agreement with Dhawanthat while regulations can be enabling provisions, but self regulation is also needed on the part of communities to meet the challenges of environmental pollution.

Mr. Bharat Singh, Ministry of Environment, Forest & Climate Change, recounted his previous association with Toxics Link during the Stockholm convention and echoed Sachdevain saying that he was here for exposure and guidance given that he is part of regulatory mechanisms and responsible with drafting mandatory standards. He also emphasised that besides drafting rules, imbibing civic sense if necessary, because community participation is required for the success of any initiative.

Satish Sinha, Associate Director, Toxics Link, thanked everyone present and said it was encouraging to see such a large gathering especially as when this meeting was being planned at Toxics Link, there were concerns on who could be invited! He highlighted that while EDCs have been clearly mentioned in the Hazardous Waste Rules, but there is not much public conversation on EDCs in India per se. While a lot of work is being done across the country, but it is not being shared adequately. And so this meeting is a platform for sharing data, experience and knowledge. It is equally a channel to identify experts, agencies,institutions as stakeholders to collectively reflect on what can we do with the information currently available.

This is a very complex issue and since India is part of a global alliance on the same, it is important that our steps are informed duly with adequate consultation and knowledge. Satishalso noted with concern the huge quantity of products in the market containing BPA which the citizens are not aware of it. How to make them aware on this issue then becomes an important question. He added that the meeting gathers people from India and neighbouring countries, some of whom have been partners with TL since the campaign on lead in paints, as a result of which, most of these countries adapted new standards. He expressed hope that this partnership would continue successfully on EDC. As a last comment he pointed out the absence of industry groups and manufacturers in the meeting, despite been given an invitation. TL however remains open to talking to them as experience suggests that over time they become more receptive to dialogue.

Technical Session 1

Dr. Geeta R. Vanage, Scientist F at the National Centre for Preclinical Reproductive and Genetic Toxicology looked at the EDCs and the impact on women's reproductive health. First, she made a review of the Epidemiology studies for EDCs in India, particularly focusing on epidemiology studies for Bisphenol A. She observed that till date very few studies exist in India which studies the association of EDCs with infertility.

The reviewed epidemiological studies have several limitations, including small sample size, limited details on subject selection criteria. She also pointed to a number of limitations regarding the analytical methods used for quantification ofEDCs and use of controls. Then she presented the study conducted at their center on determination of Plasma Levels of Endocrine Disrupting Chemicals, namely BPA, Phthalates and PCBs in fertile and Infertile Women. The objectives also include development and validation of bio-analytical methods for quantification of these chemicals in human plasma. The findings of the study would be available in a few months and would be helpful in designing future studies in specific geographical areas for understanding association of exposure to EDCs with female infertility disorders.

The study will also be helpful in determining preventive measures. Thereafter DrGeetapresented a review of Early Life Exposure to BisphenolA Impairs Reproductive Health During Adulthood. Through a detailed explanation of a study conducted on rats, she highlighted that Sensitivity to BPA is not same at all stages of life but certain critical windows are more sensitive to effects of BPA including perinatal and neonatal periods. She further pointed out the epigenetic effects of Bisphenol A on male fertility and concluded that Continuous exposure to low dose of BPA may cause adverse effects on human health which suggests the need to readdress the criteria for risk assessment.

Dr. Partho Roy, from the Department of Biotechnology at IIT Roorkee, presented on Endocrine disruptors: Their impacts on health at exposures. He said that Endocrine Disrupting Chemicals, such as phthalates, DDTs, PCBs, PEDBs, BPA are those chemicals which though are not steroids but mimics steroid. They are present in air, water, food, and a number of household products. He then illustrated the studies done at their department to understand the effects of physiological systems of animals, including reproductive failure, birth defects, developmental abnormalities, reduction and variation in sperm structures, thyroid disruption and metabolic disorders, obesity and cancer. Here is noted that the time of exposure is the most critical factor.

The study in discussion had multiple objectives. The first objective was screening of various EDCs for (anti)/estrogenic, (anti)/thyrogenic and (anti)/obesogenic actions in vitro and it was

found that among all selected compound TBTCl and TCS were found to be most effective endocrine disruptor as shown in initial screening tests.TBTCl was most harmful among all because it acted at very low dose of treatment.

The second objective was the assessments of estrogenic effect ofTBTCl and its mechanism of action in vitro and in vivo and it was noticed that TBTCl at concentrations less than human physiological level is ERα agonist and also enhances aromatase activity which portraits its estrogenic behavior; it acts through two different pathways apparently in a synergistic way; firstly, it is through ligand dependent activation via binding to ER which ultimately leads to the enhancement of steroidogenic pathways specifically through the up regulation of aromatase which is a key enzyme for estrogen synthesis; secondly, its activation of MAPK pathway showed additional activation of ER through non-genomic pathways simultaneously.

The third objective was the assessments of estrogenic effect of Tributyltin on regulation of ovarian functions in Swiss albino mice and it was noticed that TBTCl treatment resulted in an up-regulation of interaction between estrogen and estrogen receptor and altered expression of steroidogenic and steroid related proteins.Further, Serum hormonal profile and histopathological analysis revealed elevated levels of estrogen and enhanced uterine weight and wall thickness respectively, demonstrating its estrogenic potential in vivo.

The fourth objective was to assess anti-thyroidal action of TBTCl and its mechanism of action in vitro and in vivo and it was shown that TBTCl is able to modulate the thyroidal axis.Low concentrations of TBTCl suppress TR transcription by disrupting the physiological concentrations of T3/T4, followed by the recruitment of NCoR to TR, providing a novel insight into the thyroid hormone-disrupting effects of this chemical. Further, In the in vivo study, some of the genes linked to thyroid glands like TG, TPO, PAX8 etc were down- regulated in response to this chemical in mice. Lastly, the objective five was understanding the obesogenic effect of TBTCl and its mechanism of action in vitro and in vivo and it was concluded that TBTCl was found estrogenic in vitro and in vivo.TBTCl was anti-thyroidal in both in vitro and in vivo models.Obesogenic potential of TBTCl in vitro and in vivo was through multiple mechanisms including increased adipogenesis and inflammation via SIRT1 inactivation

Dr. David Gunnarson, from the Swedish Society for Nature Conservation made a presentation on EDCs: an overview of health impacts and regulation. He began by introducing his organisation, which was founded in 1909 and is one of the largest environmental NGO in Sweden which works to identify environmental threats, present innovative solutions to environmental problems and information to the public. He then delienated on the range of EDCs which are found in industrial chemicals, pesticides, cosmetics, pharmaceuticals and naturally occuring substances, which may disturb endocrine functions through different mechanims of action, such as by interfering with synthesis and/or, secretion and/orsignalling of a peptide or steroid hormone.

Through examples of international studies, he e xplained the public health effects of EDCs as how exposure has been associated with reproductive malformations and disorders, diabetes, obesity, cardiovascular disease and neuropsychological diagnoses. These chemicals have

special characteristics such that Non-monotonic dose-response relationships, whihc means a low dose may have the opposite effect compared to a high dose, timing of exposure is crucial such that windows during fetal life may by extremely sensitive, there is often a long time from exposure to effects, making it difficult to discover relationships, certain population groups may be extra vulnerable, genetic variations may increase/decrease the vulnerability significantly, the effects of exposure to an EDC is often dependent on the levels of endogenous hormones and low concentrations of individual EDCs can together cause adverse effects (also substances from varying product groups). He then explained the accumulating evidence on how EDC exposure contributes to the negative effects seen in the population.

It has been seen that exposure to certain chemicals (in animal experiments) give rise to the same type of disorders as observed in the human population, eg. obesity, affected pubertal development and hypospadias, associations between chemical exposure and these negative health effects have been found in epidemiological studies, second-generation immigrants have the same frequency of these disorders as the inhabitants of the ”new” country, accidental releases of EDC:s has been found to affect reproductive function. Further he illustrated the cost of inaction, i.e. how reducing EDC exposure reduces public expenditures through examples of US, EU, Africa and Sweden. Discussing EDC in a regulatory context, David explained the delays in making regulations in EU. He concluded his presentation through a few recommendations, such that criteria for EDC:s, that are protective enough for human health and the environment, should be established ASAP, criteria should be set on a scientific basis only (i.e. impact assessment not relevant), the use of substances listed as EDC:s by the International Chemical Secretariat (ChemSec) should be banned, national chemical restrictions should be allowed, authorities should ensure that the legislation is followed, differentiated taxes on chemicals should be introduced, making it profitable to use the least toxic ones, public procurement should demand products free from EDCs and there should be an increase the use of scientific, non-GLP, studies in the risk assessment of chemicals.

Discussion

Chair by Dr. Alok Dhawan

The first question was posed to David and he was asked to elaborate on his comment about the need to increase non GLP studies in risk assessment. In response, David clarified that although this is required, but it is an enormous amount of work and the challenge is also to incorporate these in the regulatory processes. On this point, Dhawancommented that the regulatory processes sometimes take decades to accept scientific findings and if we wait for these bodies to decide whether or not these chemicals are safe, it may be too late. So if there are GLP studies, they must be taken into account, even if they don’t fit with the demands of the regulatory agencies.

Next, Dr Geeta was asked to give some more details on the studies on infertility highlighted in the presentation. She said that these studies are done at an infertility clinic at their institution. The study she mentioned is currently in process and its results would be out in a few months. She reiterated her point about the difficulty in drawing clear associations in EDCs as it is not easy to determine time and amount of exposure. What can be said is that there is some link between elevated link of EDC and the pathology in question.

Bharat Singh asked Dr Geeta to explain the reason for increasing levels of DDT in blood, even though procurement of DDT has gone down since early 2000s across the country. He also asked her which state was her sample drawn from. Dr Geeta responded that these are persistent pollutants which are in use for a long period. Even if they were used 20 years back, they remain in the environment and people are exposed to it. It therefore becomes difficult to assess how and when someone is exposed. The amount of usage also makes an impact. At one point, DDT was the only solution available in our country and spread in high concentrations. Adding to her point, Dhawanpointed out that according to research literature, apart from direct exposure, indirect exposure of DDT through the food web is common and its traces can be found in breast milk. He also suggested that even if a compound is used in one state, it can travel through air. Excess pesticides used in one part of the planetimpacts the entire globe. And if vector control is done in densely populated areas, it is almost like pushing that in somebody's nose. He also commented on the studies and said that to make any correlation between EDCs such as BPA and human pathology, one should be able to say something about the environment they live in. Sometimes in the race of publishing papers, the scientific community forgets what should have been done to make the data tall. Studies should be precise, well planned and large, which strengthens policy makers, regulators and NGOs. If there are loopholes in the study, then the industry always comes up with ways to stay in business.

Alex asked the panel about what is the import of all the scientific data from India and the world as presented for the regulatory processes in India. She also enquired about the regulatory measures in pipeline. In response, Sunil Sharma, a government official informed that he is involved with the process of formulating a policy related to chemical standards,taking into account some global norms. And the information presented in this meeting would feed in that process. On this point, Dhawan added that given the country has about 14 out of 16 climatic conditions, adopting foreign standards serves no justice. This is a constant fight with the Bureau of Indian Standards. A proper debate on standards is needed so that we are careful with what chemicals are released in the environment. Discussion is required on not just product quality and rating but also safety issues.

Technical Session 2

Dr. Rakesh K. Tyagi, Centre for Molecular Medicine, Jawaharlal Nehru University made a presentation on the erosion of cellular transcription memory by EDCs. First he explained the concept of cellular transcription memory and nuclear receptor superfamily. Through a detailed discussion of

their study, he explained that cells utilize a process of retention and transmission of active transcription memory from progenitor to progeny via modulated transcription factors, therapeutic or chemical intervention may alter this natural process of retention and transmission cellular transcription memory and that nuclear Receptors that maintain the cell specific proteome may act as epigenetic marks. Disruption of these marks may alter the proteome of the cells leading to apoptosis or may impose upon some cells to survive dysregulation leading to uncontrolled proliferation of cells (cancer).

Dr. Ashok P. Manekar from the Molecular Endocrinology Laboratory, Department of Zoology, Institute of Science, Mumbai, presented on the effect of Sildenafil Citrate (Pink Viagra) on physiological functions in female rats. He first explained on infertility which is one element of a spectrum of reproductive disorders that include miscarriage, congenital abnormalities, premature delivery and stillbirth and other physiological abnormalities.Sildenafil, the first licensed oral treatment for male and female reproductive dysfunction has been marketed throughout the world. Also called the Pink Viagra, it is used to improve sexual desires in

women; it is not a hormone, herbal supplement or aphrodisiac. Through a detailed discussion on the study, he concluded that long term treatment of Sildenafl citrate (Pink Viagra) can cause multiple defects in physiological functions and also affects male fertility.

Discussion

Mr. Satish Sinha made an observation that these presentations have been an eye opener as the impression till now was that there is no debate on EDCs in this country. But these studies show that enough work is being done to assess the impact of EDCs on physiological functions. He asked the panellists to reflect on how this work can feed into policy advocacy processes.

In response, the panellists shared that these studies are done with an objective to generate public and policy discussion on the issues as has been done in the past on use of antibiotics or DDT. Any molecule that is released into the environment has to be done cautiously and assessed properly in labs. Their purpose is to share what is causing what effects which can be used by policy advocates for formulating regulations and taking the message out to the public.

Dr Geeta commented that mass awareness is equally important as regulation, such as in the case of DDT where despite government regulation, the farmers, who are untrained spray the chemical liberally, without proper masks or clothing. Exposure has to be given to all to avoid these chemicals as far as possible.

Technical Session 3

Dr. Prashant Rajankar, **Toxics Link** began his presentation by explaining the history and scope of work of Toxics Link, which was set up as an information, outreach and environmental advocacy organization in 1996.

The presentation focused on Bisphenol -A which is used widely in the market even as its use is contested for while the plastic industry states that the amount of BPA that a normal person is exposed to is not toxic, but other scientists and researchers have found that it causes many health problems such as Structural damage to the brain, Changes in gender-specific behavior, and abnormal sexual behavior, Increase fat formatio n and risk of obesity, Stimulation of prostate cancer cells and Hyperactivity, increase aggressiveness, and impaired learning. He explained then the Toxics Link study on BPA on children's products.

The study was done with the objective of detecting the presence of BPA in BABY FEEDING BOTTLES and SIPPERS sold in Indian market, find the claim of BPA free, to highlight the urgent need of regulation for BPA in baby products and to understand the overall consumer perspectives towards BPA and BPA free bottles in India. The results showed that 78.5% of baby feeding bottle samples contain BPA, with the maximum concentration of 9.4 ppm. Further, in 50% of BPA-free samples BPA had been detected above EU threshold limit of 0.6 ppm. Similarly, 77% samples of sippy cups contain BPA, with the maximum concentration – 14.9 ppm. In 83% (5/6) of BPA-free samples detected BPA. He concluded that the study reveals false claim of the manufacturers that their products are BPA free, presence of consumer demand towards the BPA free products. He also recommended such that the children's products needed to be strictly regulated, should be addressed, India should take some affirmative action to regulate the use of BPA and the Government of India needs to come out with a research agenda to identify the source contamination of BPA from various products and its impact on human health.

Prof. S. B. Deshpande, Department of Physiology, Institute of Medical Sciences, Varanasi also presented on Bisphenol A, specifically the toxicity induced by this compound in vital organs of rats. He began by discussing on Bisphenol A, its nature and use and points of exposure such as through ingested food, inhalation in the form of dust, skin absorption, and food packaging. Infants and children are particularly vulnerable through intake of breast milk due to maternal exposure. BPA is also found to have an impact on reproduction, mammary gland, prostate, urinary tract, developmental toxicity and behavioral alterations. They conducted a study to ascertain the acute and chronic effects of BPA and plastic boiled water (PBW) on lung, heart, liver, kidney and uterus.

The experiments showed that chronic ingestion of plastic leached water produced severe emphysematous changes in lung and PBG-induced cardio-respiratory responses were attenuated in these rats. Thereafter, acute experiments were performed with BPA to understand the mechanism of attenuation of reflexes. What was found that chronic exposure to plastic leached water produced emphysematous and consolidative changes in the lungs. The pulmonary pathology may be due to the chronic attenuation of the protective respiratory reflexes by Bisphenol A and Plastic leached water treated animals exhibited disruption of the cardiac muscle fibres and broken fibrils at intercalated disc. When acute experiments were performed to understand the functional changes in cardia, the results showed structural changes associated with BPA or plastic water on heart. The observations also demonstrated that BPA depressed the contractility of spontaneously beating atria *in vitro*. Further the involvement of NO-guanylylcyclase pathway for the BPA-induced decrease in atrial contractions.

Alexandra Caterbow, Co-chair of IPEN EDC working group presented on the global initiatives to regulate EDCs. First she briefed on the IPEN EDC working group which works in cooperation with Endocrine Society and is involved in advocacy work in SAICM process, capacity building and awareness raising. She mentioned the WHO/UNEP Report "State of Science of EDCs – 2012", which was the first overview of international research results on EDCs with chapters on definition, harmful effects on humans, wildlife and environment. EDCs is now an emerging policy issue in SAICM with regional resolutions in Latin America, Asia and Africa, which address the topics of **vulnerable groups**, **pesticides**, **identification of priority EDCs**, **EDCs in products**, **capacity needs of stakeholders**, **human bio-monitoring**, **safer alternatives**, and **cost of inaction**. They recommend organizing a series of awareness raising and capacity building activities. She highlighted details on the UNEP projects on EDCs and the EDC regulation at EU who'sREACH covers certain EDCs, Pesticides Regulation, Biocides Directive, Toys Directive, Cosmetics Directive and Pregnant women at the workplace directive. She underlined that while there are a number of studies financed by public money which prove that BPA is harmful even in low doses but there are no EU laws which prohibit BPA and while a number of products contain BPA but no products containing BPA have been prohibited in Europe. She explained the delays and diversions in the EU process. Further she highlighted the health costs in EU related to EDCs per year. She concluded her discussion by showing the information available for vulnerable groups in countries like Denmark and Austria, France and Spain etc.

Prof. Surya Pratap Singh, **Department of Biochemistry at University of Varanasi** made a presentation on the deleterious effects of Bisphenol A on fertility and cognition of male mice and its alleviation by *Mucunapruriens*. Following an introduction to the compound and the associated risks, he explained his study which was done to understand the protective effect of *Mucunapruriens*on BPA afflicted male infertility and cognitive dysfunctions. The results from the present study strongly suggest that BPA has deleterious effects on reproduction of adult male mice. Significant decline in the level of testosterone reveals the steriodogenic disruption caused by BPA. The mode of action of BPA can be contributed to the increased expression of iNOS which further leads to a reduced expression of StAR

thereby affecting the steriodogenesis process. Further, L-DOPA content of the plant helps to increase the testosterone level and helps to improve the spermatogenic and steroidogenic activity andthat an increase in sperm count and motility. Also MP helps to improve the DAT and NMDAR expression in the hippocampal region of mice brain which helps in improving the performance of the mice at spatial navigation tasks.

Discussion

Chair by Mr. Ashim Sanyal

Girija from USAID asked the panel about whether citizens can trust the industry when it labels certain products as BPA free, and does the scientific community endorse the same. In response, Ashim noted that BPA labellingby industry is voluntary, so it is only a claim, which can of course be tested.

Prashant asked a question to Alexandra about how contradictions are managed and convergence ensured between different government departments in Europe. She replied that contradictions and differences between departments is a big problem in Europe as well. In such a scenario, all that advocates can try and do is support allies and convince non allies. As such, voluntary measures don't work; what works are incentives or legal measures, which need to be pushed for.

Satish asked a question to Alexandraon when a single country (e.g. Denmark) comes up with standard, how it sources materials which are not mass produced and therefore more expensive. Alexandra responded that when a country take a strong stand by making national standards for public health, often other countries follow and if one or two member states say they don't want these products, then it becomes easier for manufacturers to producealternatives. She added however that as such even if we replace plastic containing BPA with a better version, it is still plastic, which has a very long life within the eco system. Best solution therefore is to not use plastic.

Pannel Discussion

Mr. Sinha from Toxics Link, began the panel discussion with an observation that the presentations made in the day gave a fairly good idea of how EDCs impact human health but the environmental impacts of these compounds also need to be discussed. He also asked everyone to respond briefly to the question on what kinds of mechanisms should be put in place to regulate this market.

Ashim said that in a country like India, which is still grappling with feeding its people and ensuring food fortification, it is natural that preventive measures like regulating EDCs take a back seat. And as such, Indians are least bothered about the environment. Therefore, as their experience with the national campaign 'JagoGrahakJago' shows, awareness building has a rather slow impact.

Mr. Suman Basu, from Estman co. said that consumers should also make an effort to know the technical labelling on products (such as on recycled plastic bottles) and purchase those that are better for the environment. Adding to this point, Mr. Bharat Singh noted that health consciousness is imperative in the country, for unless we reach hospital beds, we don't realise the implications of being ill informed. Dr Geetaalso continued on this thread and said that people need to be educated

for minimum and proper usage of hazardous chemicals. She also emphasised on the need for systematic epidemiological studies on EDCs such as those carried out in the west. These must be especially done in areas where pesticides are used regularly. This is important so there is solid data to show to the government and pressurise the policy makers for action. In this regard, agencies that could fund these studies should also be identified.

Mr Sachdeva said that EDCs is a complex issue as the evidence most available is not direct but an associative one. This is why industry lobbies and beneficiaries always raise questions on the data. Also, there are very few civil society groups working in the field. The group should therefore think of how it can emerge as an active pressure group to advocate with the government. The way forward should also be to look for donors to fund this research. Responding to this, Ashim said that CSR units of companies may be approached for such research grants. A suggestion also came up on asking Ministries for financial assistance to research institutions and NGOs.

Suggestions also came in targeting schools and young people for awareness building. On this point, David pressed that there is no contradiction between soft and hard measures, as is often viewed. For example, soft measures such as information campaigns may pave the way for voluntary phase out by corporations, enabling further legislations. The opposite may also happen that normative legislative frameworks change peoples' mindsets and opinions. Alex commented that awareness building is a slow process and consumers are often without much information on what they should buy whether in India, Germany or elsewhere. But what is really needed is a good regulation. Here she suggested that if scientists get some research funding, they should reserve a little money for NGOs to work on bringing that information to policy makers. Often good research is published but fails to make it to the next step. She also noted that the situation here is parallel or similar to that in Europe and other contexts in terms of search for research funds, ping pong between institutions etc. What one needs is individuals in key positions who feel responsible for this and who are willing to drive it forward or else things will be same after 10 years.

Building up observations made by Alexandra, participant from Nepal asked the group whether it is feasible to deal with one chemical at a time because as new chemicals come in focus, it essentially means a repetition of the entire policy advocacy process. Is it possible to think of a comprehensive chemical safety policy which allows a wider window to include more chemicals?

Responding to the discussion thus far, Satish asked the group to deliberate on how to make this subject a priority with the government and if that can be done by focusing on the cost of inaction, i.e. how the continued use of chemicals would financially bear on disease and treatment. He also raised an important question of whether one needs a national data for everything. Given that so much research literature is available across the world, is it required to reinvent the wheel, and why is the globally available data not acceptable to the Indian government and industry?

Concluding the discussion for the day, Satish summed up the main points.

- Public information should be a focus because that helps to build a critical mass that can initiate change.
- We should think of rewards or incentives for companies or groups engaged in voluntary phase out of hazardous products or making products that are BPA free.

- Governments need to take into account the economic costs of disease control in the debate on policy options.
- We need to focus on finding mechanisms and platforms for bridging the gap between scientific community and the industry, such as through an interdisciplinary research consortium. We need to convert scientific studies into social goods towards the making of a comprehensive policy.

Presentation are apended bellow

EDCs and its health impacts on women's reproductive health (Indian context)

Dr. Geeta R. Vanage
Scientist F

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ENDOSULFAN TRAGEDY

- **CONGENITAL ABNORMALITIES:** Congenital heart and skeletal abnormalities were also high
- **NEUROLOGICAL PROBLEMS:** learning disabilities, low IQ
- **ABNORMALITIES IN REPRODUCTIVE SYSTEM:** menarche early, menstrual disorders, decrease in sperm count, defects in spermatogonial cells, sperm morphology and other defects in male sex hormones

over-growth of head

Epidemiology studies for EDCs in India

Authors & Year	Study type	Measurement of EDCs	Outcome
Siddiqui et al 2003	mothers with IUGR babies (n=30) and from those with babies of normal weight (n=24)	Persistent chlorinated pesticides were estimated maternal blood, placenta and cord blood, collected at parturition using GC	Significant associations between maternal blood levels of alpha-HCH, gamma-HCH, delta-HCH, total HCH and p,p'-DDE and IUGR
Pathak et al 2009	case-control study: 23 cases each of preterm labor and full term labor were	Organochlorine pesticides were estimated in Maternal and cord blood samples using GC	Significant relation was observed between preterm birth and beta-HCH levels
Pathak et al 2010	Recurrent miscarriages cases and women with normal full term delivery with live birth (controls)	Organochlorine pesticides were estimated in blood samples using GC	Significant association was observed between blood gamma-HCH levels and women with recurrent miscarriages
Pathak et al 2010	case-control study: 30 women in each group of full-term delivery (FTD; and preterm delivery (PTD)	Organochlorine pesticides were estimated in maternal and cord blood of women using GC	↑ levels of beta-HCH and alpha-endosulfan and increased oxidative stress in PTD than FTD cases.

Epidemiology studies for EDCs in India

Authors & Year	Study type	Measurement of EDCs	Outcome
Sharma et al 2012	Case-control study: 50 fetal growth restriction cases and equal number of normal pregnancies who were occupationally non exposed to OCPs	Organochlorine pesticides were estimated in Maternal and cord blood samples using GC	Significant association was seen between β-HCH and GSTM1-genotype with reduction in birth weight of 213g
Dewan et al 2013	Case-control study: 60 infant-mother pairs, comprising of 30 term, small for gestational age babies with their mothers (Case group), and another 30 term, appropriate for gestational age babies with their mothers (Control group)	Organochlorine pesticides were estimated in maternal blood, cord blood, placenta and breastmilk samples, using GC	The OCP levels case group were ↑ than the control group; these were significantly ↑ for t-HCH in cord blood and breastmilk; β-HCH in maternal blood, cord blood and breastmilk; DDE in placenta and DDT in breastmilk

Epidemiology studies for EDCs in India

Authors & Year	Study type	Measurement of EDCs	Outcome
Tyagi et al 2015	preterm birth cases (n = 50) and subjects of term delivery as controls (n = 50)	Concentrations of Organochlorine pesticides (OCPs) were measured using GC in Maternal blood and placenta samples	Significantly ↑ levels of α-HCH, β-HCH, DDD and DDE were found in maternal blood of PTB cases as compared to control. Significantly ↑ levels of DDE and DDT were also found in placental tissue of PTB cases as compared to control group

Epidemiology studies for EDCs in India

Authors & Year	Study type	Measurement of EDCs	Outcome
Reddy et al. 2004	Prospective case-control study 85 women of with minimal–severe (stage I–IV) endometriosis and 135 with proven fertility	Phthalates & PCBs were measured using GC in blood plasma samples	No significant differences in mean plasma concentrations of the 14 PCB congeners and 11 chlorinated pesticides was observed among case and control group
Reddy et al. 2006	Case–control study.: Study group: 49 infertile women with endometriosis Control group I: 38 age-matched women without endometriosis but with other infertility related defects, Control group II: 21 age-matched women with proven fertility	Phthalates were measured using GC in blood samples	Study group showed significantly ↑ concentrations of DnBp, BBP, DnOP and DEHPcompared with control group I and II

Epidemiology studies for EDCs in India

Authors & Year	Study type	Measurement of EDCs	Outcome
Rozati et al 2008	Prospective case-control study: study group: 99 infertile women with endometriosis control group: 135 age-matched omen without endometriosis	Phthalates were measured using HPLC in blood samples	Significantly ↑concentrations of DMP, DEP, DnBP, BBP & BEHP were found compared to controls

Epidemiology studies for Bisphenol A

Authors	Study Participants	Measurement of BPA	Health related outcome
Kuroda et al., 2003	Cross-sectional: 9 healthy pregnant women, 21 women with sterility	BPA levels in serum and cord blood from pregnant women; serum and peritoneal fluid from women with sterility (measured by HPLC)	Sterility
Hiroi et al., 2004	Cross-sectional:7 women with endometrial carcinoma, 9 women with complex endometrial hyperplasia, 10 women with simple endometrial hyperplasia, 11 controls	Serum BPA levels (by ELISA)	Endometrial carcinoma and hyperplasia
Sugiura-Ogasawara et al., 2005	Cross-sectional:45 women with recurrent miscarriage and 32	Serum BPA levels (by ELISA)	Recurrent miscarriage

Epidemiology studies for bisphenol A

Authors	Study Participants	Measurement of BPA	Health related outcome
Hanaoka et al., 2002	Cross-Sectional: 82 subjects (42 epoxy resin sprayers and 42 unexposed to BADGE)	Urinary BPA levels (by HPLC) in workers applying epoxy resins and unexposed workers	FSH levels
Takeuchi & Tsutsumi, 2002	Cross-sectional: 14 healthy women, 16 women with PCOS and 11 healthy men	Serum BPA levels (by ELISA)	PCOS
Yamada et al., 2002	Case control: 48 cases with abnormal karyotype, 200 controls (20 per year) selected from women carrying fetuses with normal karyotypes	BPA levels in maternal serum and amniotic fluid at time of amniocentesis (by ELISA)	Fetus with abnormal karyotype

Lacunae

- In India till date very few studies have been conducted to find out the association of EDCs with infertility
- These epidemiological studies have several limitations, including small sample size, limited details on subject selection criteria
- Although isotope dilution methods based on mass spectrometry (MS) and tandem mass spectrometry (MS/MS) are the most reliable for the detection of EDCs, many of the results of EDCs determination in biological samples have been generated by non-MS-based methods.
- Limitation to Analytical Methods used for quantification of EDCs
  - Cross contamination during sample collection or storage
  - Poorly described sample preparation and analytical methods
  - Lack of validated Analytical Methods and proper use of controls

10

Determination of Plasma Levels of Endocrine Disrupting Chemicals In fertile and Infertile Women

Objectives

- To develop bio-analytical methods for quantification of BPA, Phthalates and PCBs in human plasma
- To validate the bio-analytical methods for quantification of BPA, Phthalates and PCBs in human plasma
- To determine the plasma levels of BPA, Phthalates and PCBs in infertile women and correlate them with female infertility.

Study Participants

Total No. of Participants: 200

Fertile Women Group = 100

Total No. of Participants recruited: 97

Infertile Women Group = 100

Total No. of Participants recruited: 48

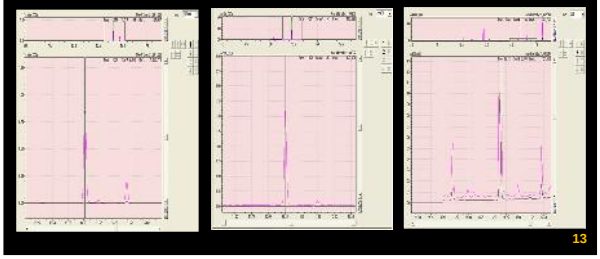
Polycystic Ovarian Syndrome: 32
Endometriosis: 13
Polycystic Ovarian Syndrome + Endometriosis: 3

12

Contamination of BPA and Phthalates In Blank Plasma and their Reduction with Activated Charcoal

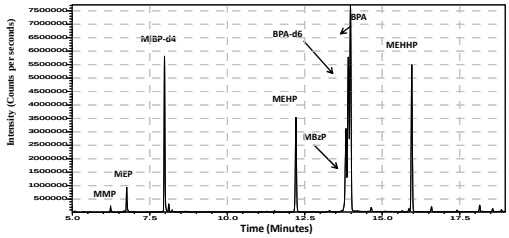
Blank Plasma without Treatment
Blank Plasma with Treatment of Charcoal

Bisphenol A Quantifier Ion- 357	MEHP Quantifier Ion- 239	MEHHP Quantifier Ion- 221



13

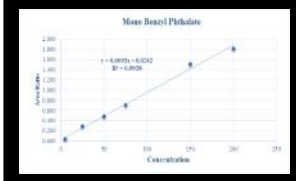
Chromatogram of Standard Mixture of Bisphenol-A, Phthalate Metabolites & Their Internal Standards Spiked in Human Plasma

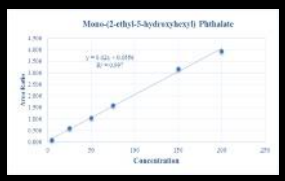


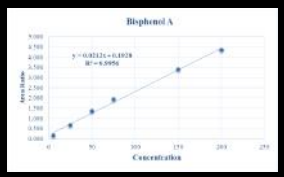
MMP- Mono Methyl Phthalate, MEP- Mono Ethyl Phthalate, MiBP-d4- Mono Isobutyl Phthalate-d4
MEHP- Mono-(2-ethylhexyl) phthalate, MBzP- Mono-Benzyl phthalate, BPA- Bisphenol A, BPA-d6- Bisphenol A-d6 MEHHP Mono-(2-ethyl-5-hydroxyhexyl) Phthalate

14

Linearity for Spiked plasma standards of Bisphenol-A, Phthalate Metabolites







15

Expected Outcomes

- Validated GC-MS methods has been developed for BPA and Phthalates which can be utilized for future studies
- The data from the study will provide preliminary information on:
  - Plasma levels BPA, Phthalates and PCBs in infertile women
  - Possible association of BPA, Phthalates and PCBs with female infertility
- The findings from this study will be helpful in designing future studies in specific geographical areas for understanding association of exposure to EDCs with female infertility disorders
- The study will also be helpful in determining preventive measures

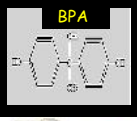
16

Early Life Exposure to Bisphenol A Impairs Reproductive Health During Adulthood

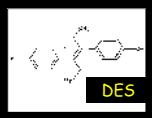
Bisphenol A(BPA)…A Historical Review

First synthesized in 1891

BPA



Structural Similarity with DES



DES

Polymer chemists discovered that it could act as a good plasticizer to make polycarbonate plastic, epoxy resins. It is used in number of consumer and house hold products,Dental selient,thermal papers etc.

BPA Levels in Biological Samples & Environment

Maternal plasma: 0.3-18.9 ng/ml
Fetal plasma:0.2-9.2 ng/ml
Placental tissue: 1.0-10.4 ng/g
Umbilical cord blood:0.62±0.1ppb
Urine samples:13ppb (93% people)

Surface water: 0.0005-0.41 mg/l
Sewage effluent: 0.018-0.702 mg/l
Sediments: 0.01-0.19 mg/kg
Sewage sludge: 0.004-1.363 mg/kg
Landfill leachate: 269 mg/l
Geographical Measurements

Ubiquitous exposure to BPA

Calafat et al,2005; Schonfelder et al,2002 ; Sheehan 2000; Kolpin et al., 2002; Staples et al., 2004; Rippen et al., 1999; Klecka et al,2001

Major concern for effect of BPA on humans is with exposure to low and environmentally relevant doses viz.2.4 µg/kg bw

Dose Dependent

BPA Effects

Life-stage Dependent

Sensitivity to BPA is not same at all stages of life , certain critical windows are more sensitive to effects of BPA

Perinatal Period

" A Pivotal Window of Reproductive Development "

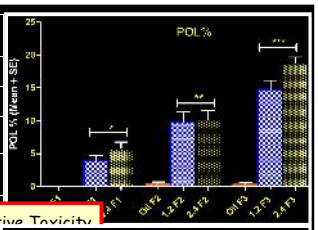
Gestation | Lactation | Perinatal Exposure | Weaning

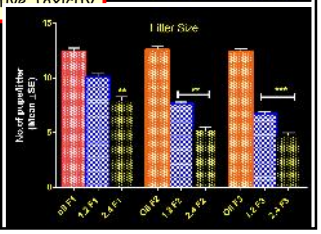
GD 0 | GD 12 | PND 0 (Delivery) | PND 21

- Encompasses crucial period of gestation and lactation
- Critical period for sexual differentiation and development of reproductive tract

GD- Gestation Day PND – Postnatal day

N=24 males/ group/ generation	Copulated females / female showing resorptions		
	F1	F2	F3
Control	24/0	23/1	24/1
1.2 µg BPA	20/8**	20/17***	20/17***
2.4µg BPA	21/6*	20/19***	20/20***
DES	6/6 ***	7/5*	





Reproductive Toxicity

No significant changes in

PIL %
Copulation index

*p < 0.05, ** p < 0.001, *** p < 0.0001 (with respect to vehicle control)

Salian et al, 2009, Life Sciences

Conclusions from Perinatal Model

BPA exposure

Impairments in fertility and spermatogenesis in F1- F3

Significant in Sperm count and motility in F1-F3

Significant perturbations in AR, ER β and SRC-1 (localized on Sertoli cells)

BPA can act as a potential Sertoli cell toxicant

Difficult to speculate the critical time frame during which BPA adversely affects HPT axis

Neonatal Window

" A Critical period of development within the perinatal window "

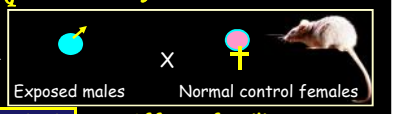
Neonatal Period in Rat
(Post Netal Day 1-5)

Neonates are at a higher risk of ED exposure than adults due to :

- Decreased metabolizing efficiency
- Limited serum binding proteins to bind to exogenous chemicals
- Akins to the second trimester of human pregnancy
- Programming of the HPT axis occurs
- Important developmental changes in testis (Sertoli cell multiplication) and reproductive tract occur or are initiated

Neonatal exposure to BPA lead to …..

Neonatal → Exposure Day 1-5



Group n= 24 males	No of females cohabitated	Copulated females / female showing resorptions
Oil	48	46/1
DES	48	15/14***
0.6 µg BPA	48	46/1
2.4 µg BPA	48	44/18***
5 µg BPA	48	42/18***
10 µg BPA	48	33/15***

Affects fertility

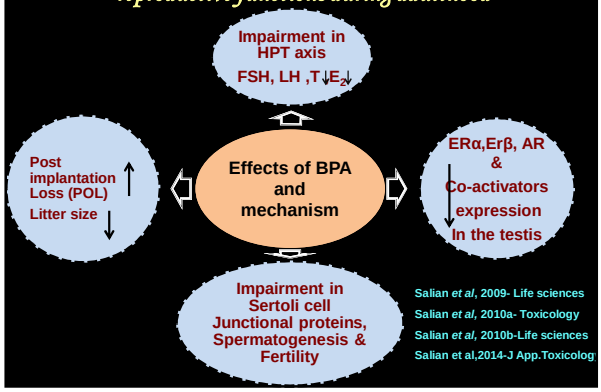
- Litter Size
- Daily sperm production
- Epididymis Sperm Count

Impairment in Hormonal profile LH↓ T↓ FSH →

*** $p < 0.0001$

Salian et al., 2009, Toxicology

Exposure of Neonatal Male Rats to Bisphenol A impairs reproductive functions during adulthood



Salian *et al*, 2009- Life sciences
Salian *et al*, 2010a- Toxicology
Salian *et al*, 2010b-Life sciences
Salian et al,2014-J App.Toxicolog

The effects of Neonatal /Perinatal Exposure of male rats to Bisphenol A on reproduction during adulthood and mechanism of its action

1. Salian S, Doshi T, Vanage G (2009) Neonatal Exposure of Male Rats to Bisphenol A Impairs Fertility and Expression of Sertoli Cell Junctional Proteins in the Testis. Toxicology 265: 55-67 [IF: 3.6].
2. Salian S, Doshi T, Vanage G (2009) Impairment in protein expression profile of testicular steroid receptor coregulators in male rat offspring perinataly exposed to to Bisphenol A. Life Sciences 85 : 11-18 [IF: 2.58].
3. Salian S, Doshi T, Vanage G. (2009) Perinatal exposure of rats to Bisphenol A affects fertility of male offspring. Life Sciences 88:742-752 [IF: 2.58].
4. Salian S, Doshi T, Vanage G. (2010). Perinatal exposure of rats to Bisphenol A affects fertility of male offspring – An Overview. Reprod. Toxicol. 31(3) : 359-362,. [IF : 3.367].
5. Salian S, Doshi T, Vanage G. (2010).Bisphenol-A affects male rat fertility for generations. Life Sciences Environmental Health News, 1-3, 2010 (IF: 2.718).
6. Salian-Mehta S, Doshi T, Vanage G. (2014). Exposure of neonatal rats to the endocrine disrupter Bisphenol A affects ontogenic expression pattern of testicular steroid receptors and their coregulators. J. Appl.Toxicol. 34(3):307-18.

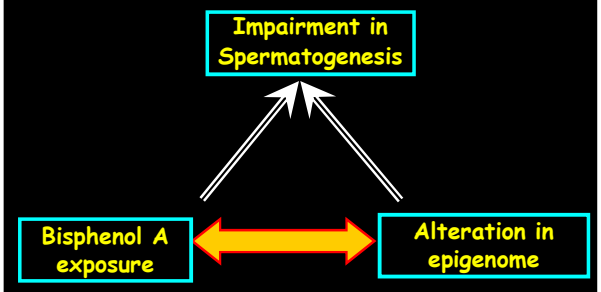
"Epigenetics" At a Glance

Epigenetics is defined as "the study of processes that produces heritable changes in genome that occur without a change in DNA sequence"

Epigenetically Regulated Processes

- Gene expression
- Genomic imprinting
- Carcinogenesis

(Fujita *et al.*, 1997; Hanada *et al.*,1993; Verma 1993; Singal,2008)



Epigenetic Effects of BPA at the Testicular Level and Mechanism of its Action

7. Doshi T, Salian S , Dighe V , Balasinor N, Vanage G. (2011). Hypermethylation of Estrogen Receptor Promoter Region in Adult Testis of Rats Exposed Neonatally to Bisphenol A. Toxicology. 289: 74-82 [IF: 4.0]
8. Doshi T, Salian S, Dighe V, Balasinor N, Vanage G. (2011). Genes change message after newborn rats given BPA. Environ. Health News 1-4. (IF : 2.718).
9. Doshi T, D'Souza C, Dighe V, Balasinor N, Vanage G. (2012). Effect of neonatal exposure of male rats to Bisphenol A on the expression of DNA Methylation machinery in the Post Implantation Embryo. J. Biochem. Mol. Toxicol. 26(9) : 337-343. [IF : 2.5]
10. Doshi T, D'Souza C, Vanage G. (2013). Aberrant DNA Methylation at Igf2-H19 Imprinting control region in spermatozoa upon neonatal exposure to Bisphenol A and its association with post implantation loss. Mol Biol Rep. 2013 Aug;40(8):4747-4757. [IF:2.7]

Significance of the Study

- Delineates the epigenetic mechanisms of BPA induced adverse effect on fertility
- Strengthens the premise that abnormal epigenetic reprogramming of the male germ line as one of the possible mechanism of compromised male fertility

Continuous exposure to low dose of BPA may cause adverse effects on human health; suggesting the need to readdress the criteria for risk assessment

- Salian S, Doshi T, Vanage G. (2010).Bisphenol-A affects male rat fertility for generations. Environmental Health News, 1-3, 2010 (IF: 2.718).
- Doshi T, Salian S, Dighe V, Balasinor N, Vanage G. (2011). Genes change message after newborn rats given BPA. *Environ. Health News 1-4. (IF : 2.718).*
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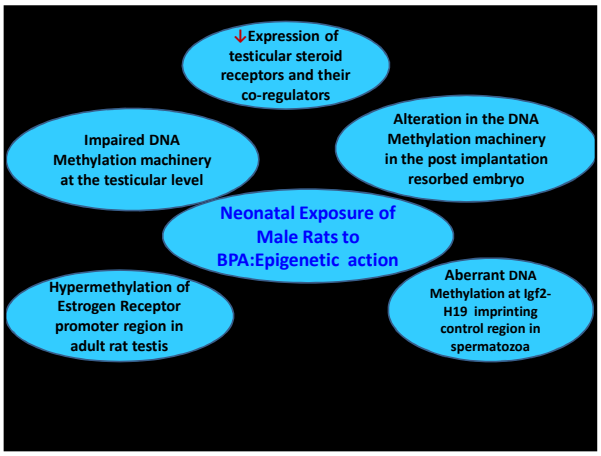
Acknowledgments

- Indian Council for Medical Research (ICMR)
- Department of Science and Technology (DST)
- Department of Biotechnology (DBT)



Genetic toxicity of BPA and mechanism of action

11. Tiwari D, Kamble J, Chilgunde S, Patil P, Maru G, Kawle D, Vanage G. (2012). Clastogenic and Mutagenic Effects of Bisphenol A, an Endocrine Disruptor Mutational Research. Genetic Toxicol. & Environ. Mutagenesis Mar 743 (1-2):83-90. [IF : 2.9]
12. Tiwari D. and Vanage G. (2013). Mutagenic effect of Bisphenol-A on adult rat male germ line and their fertility. J. Reprod. Toxicol. 40 : 60-68.

INDIAN INSTITUTE OF TECHNOLOGY ROORKEE

Endocrine disruptors: Their impacts on health at exposures

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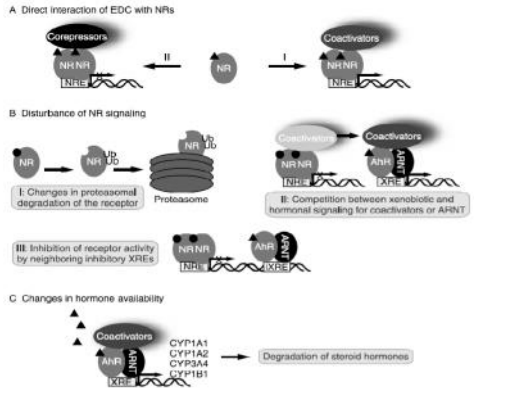
EDCs : Endocrine Disrupting Chemicals are those chemicals which though are not steroids but mimics steroid

IIT ROORKEE 2



Adopted from: Swedenborg et al., Journal of Molecular Endocrinology (2009)

Real-life look of EDCs

5

Effects on physiological systems of animals

- Reproductive failure
- Birth defects
- Developmental abnormalities
- Reduction and variation in sperm structures
- Thyroid disruption and metabolic disorders
- Obesity
- Cancer

Timing of exposure – a critical factor

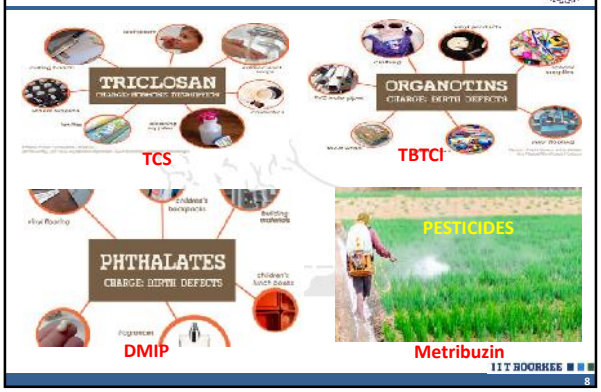
IIT ROORKEE 6

OBJECTIVE 1

Screening of various EDCs for (anti)/estrogenic, (anti)/thyrogenic and (anti)/obesogenic actions in vitro

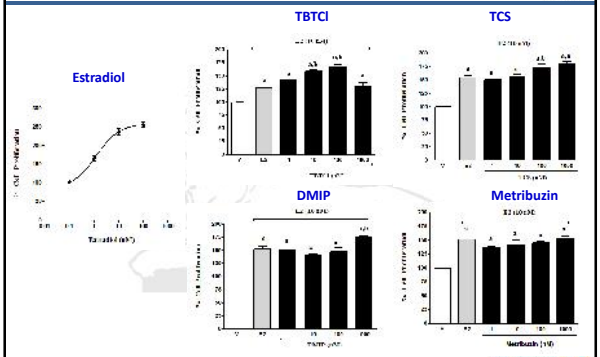
IIT ROORKEE 7

A Brief Introduction of test Chemicals



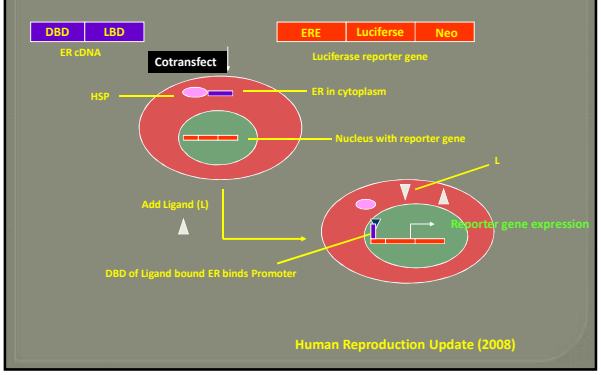
IIT ROORKEE 8

E-Screening assay (MCF-7 cells)

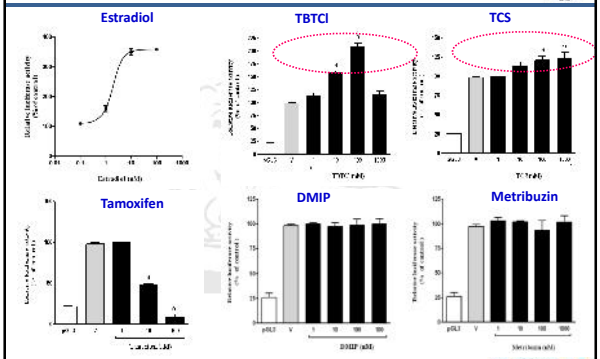


IIT ROORKEE 9

Scheme of Transactivation assay

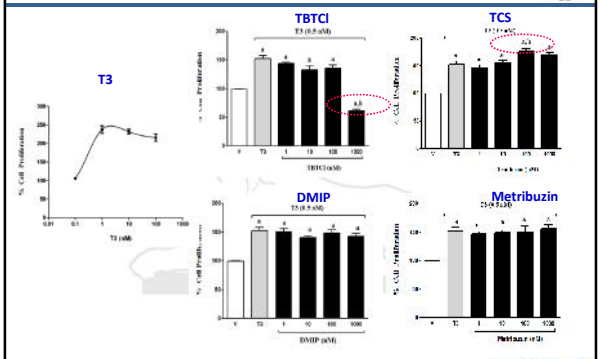


Transactivation assay (MCF-7 cells)



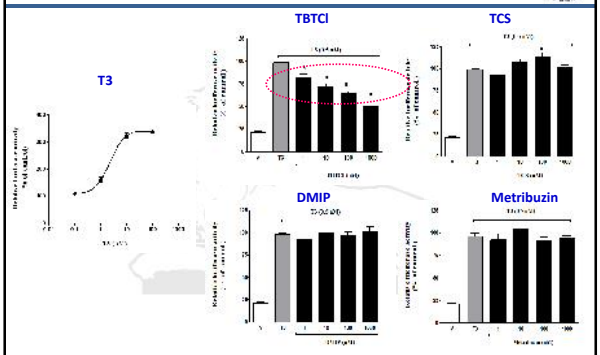
IIT ROORKEE 11

T-Screening assay GH3 cells



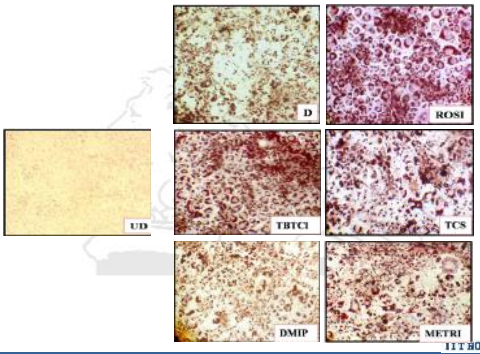
IIT ROORKEE 12

Transactivation assay (GH-3 cells)



Adipocyte differentiation assay (3T3L1 cells)

Oil-O-Red staining



Background

Members of the nuclear hormone receptor superfamily

PPAR

Functions

Adipogenesis
Lipid and glucose metabolism
Immune function
Cell growth
Cell Differentiation

Disease

Obesity
Diabetes
Cardiovascular disease

Cancer

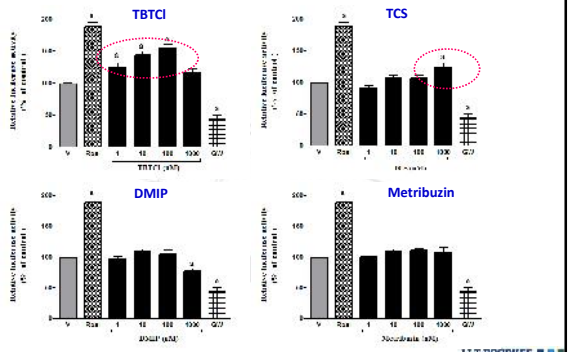
PPAR

- **PPARα** — liver, kidney, heart, muscle, adipose tissue
- **PPARβ** — Brain, adipose tissue and skin
- **PPARγ** — Heart, muscle, colon, kidney, pancreas and spleen

PPARγ — **Most promising biomedical molecules in the 21st century**

- PPARγ is expressed strongly in adipose tissue and is a master regulator of adipocyte differentiation
- PPARγ is also expressed in tissues such as breast, colon, lung, ovary, prostate and thyroid, where it regulates cell proliferation, differentiation and apoptosis

PPARγ Transactivation assay (3T3L1 cells)



CONCLUSIONS

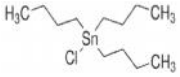
- Few EDC that we encounter in our day today life were evaluated for their (anti)/estrogenic, (anti)/thyrogenic and (anti)/obesogenic activities in different model system.
- Among all selected compound TBTCl and TCS were found to be most effective endocrine disruptor as shown in initial screening tests.
- TBTCl was most harmful among all because it acted at very low dose of treatment.

OBJECTIVE - 2

Assessments of estrogenic effect of TBTCl and its mechanism of action in vitro and in vivo

A Brief Introduction of test Chemical

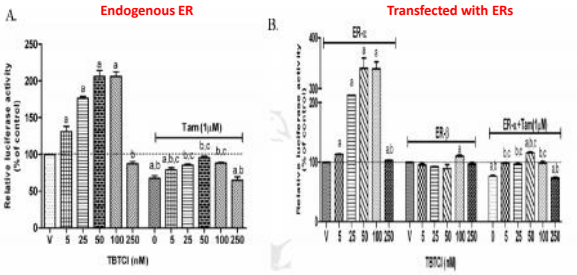
Organotin- TBTCl



Organotins, specially Tributyltin chlorides (TBTCl) compounds are a group of compounds containing the $(C_4H_9)_3Sn$ moiety and used in wood preservation, antifouling pesticide in marine paints, antifungal action in textiles and industrial water systems, such as cooling tower and refrigeration water systems, wood pulp and paper mill systems, and breweries.

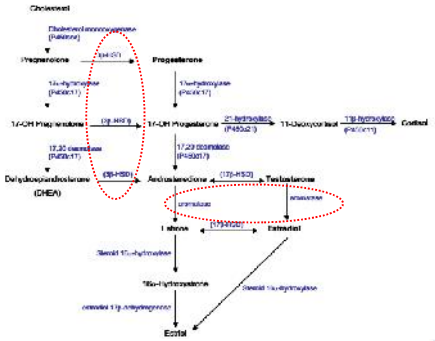
Effects of low dose treatment of tributyltin on the regulation of estrogen receptor functions in MCF-7 cells

ER Transactivation assay in MCF-7 cells

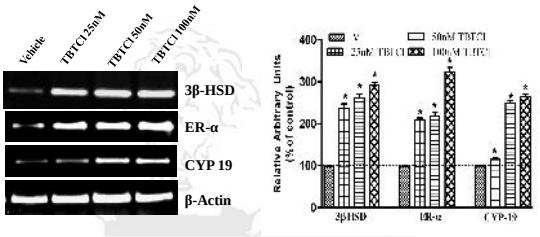


Sharan et al., 2013 *Toxicol Appl Pharmacol.* 269(2):176-86.

Steroidogenic pathways and important enzymes Involved

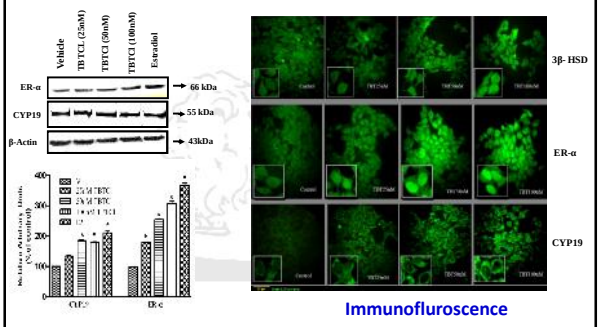


Transcriptional analysis of various steroidogenesis related genes



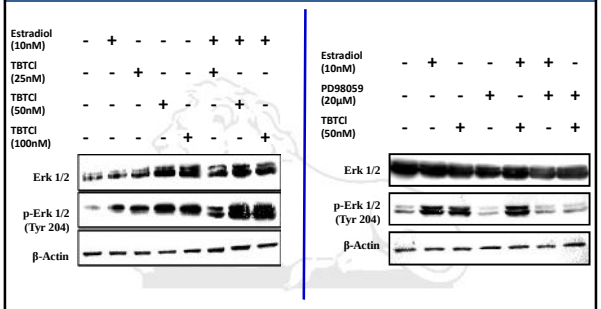
Sharan et al., 2013 *Toxicol Appl Pharmacol.* 269(2):176-86.

Translational analysis of CYP19 and ERα protein in MCF-7 cells



Sharan et al., 2013 *Toxicol Appl Pharmacol.* 269(2):176-86.

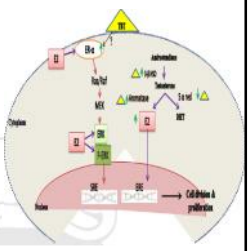
Phosphorylation of ERK1/2 and its role in non-genomic action of Estrogen



Sharan et al., 2013 *Toxicol Appl Pharmacol.* 269(2):176-86.

CONCLUSIONS

- TBTCl at concentrations less than human physiological level is ERα agonist and also enhances aromatase activity which portraits its estrogenic behavior.
- It acts through two different pathways apparently in a synergistic way.
- Firstly, it is through ligand dependent activation via binding to ER which ultimately leads to the enhancement of steroidogenic pathways specifically through the up regulation of aromatase which is a key enzyme for estrogen synthesis.
- Secondly, its activation of MAPK pathway showed additional activation of ER through non-genomic pathways simultaneously



OBJECTIVE – 3

Assessments of estrogenic effect of Tributyltin on regulation of ovarian functions in Swiss albino mice

Animal Experimental design

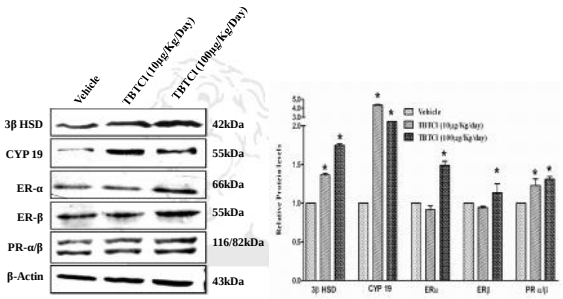
N=18 female mice
(6 each group)
20 days old

- Vehicle
- TBTCl (LD) (10 μg/Kg)
- TBTCl (HD) (20 μg/ Kg)

Gavaged for 45 days

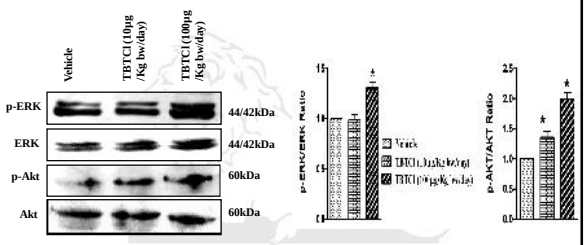
Mice Sacrificed at the end of trial and blood and organs were collected

Protein expression of steroidogenic and estrogenic genes in the ovary of TBTCl treated mice



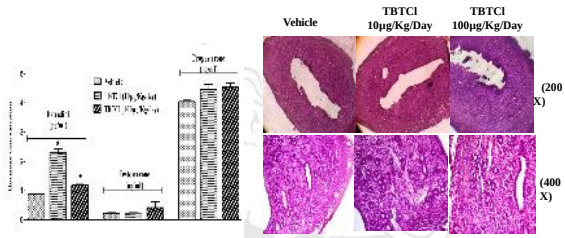
Sharan et al., 2015 (Under Revision in *Reprod. Toxicol.*)

The effect of TBTCl in proliferation and the regulation of signaling pathways in the ovary



Sharan et al., 2015 (Under Revision in *Reprod. Toxicol.*)

Serum hormone level and Uterine Histology



Sharan et al., 2015 (Under Revision in *Reprod. Toxicol.*)

Hematological Parameters

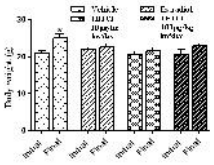
Parameters	Control	TBTCl (10µg/kg bw/day)	TBTCl (100µg/kg bw/day)
Hemoglobin (g/dL)	12.68±0.52	14.28±0.14*	15.88±0.63*
Red blood cells (mm³)	9.46±0.24	11.27±0.37*	11.98±0.45*
White blood cells (/mm³)	7.8±1.05	6.6±1.47	6.16±0.51*
PCV (mm)	50.22±1.79	51.46±0.71	50.68±1.59
Neutrophil (%)	19.12±0.19	18.46±0.71	19.68±1.09
Lymphocyte (%)	78.28±1.14	77.22±3.33	75±3.12

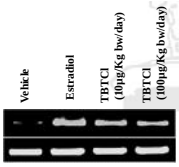
Sharan et al., 2015 (Under Revision in *Reprod. Toxicol.*)

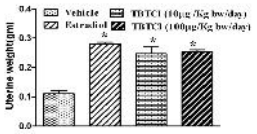
Organization for Economic Cooperation and Development (OECD)

Uterotrophic assay For evaluation of TBTCl estrogenicity

Changes in Uterine weight



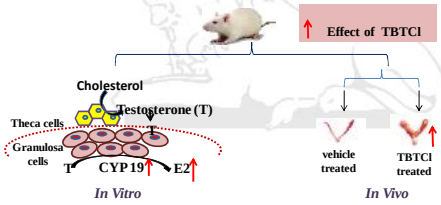




Sharan et al., 2015 (Under Revision in *Reprod. Toxicol.*)

Conclusions

- TBTCl treatment resulted in an up-regulation of interaction between estrogen and estrogen receptor and altered expression of steroidogenic and steroid related proteins.
- Serum hormonal profile and histopathological analysis revealed elevated levels of estrogen and enhanced uterine weight and wall thickness respectively, demonstrating its estrogenic potential *in vivo*.

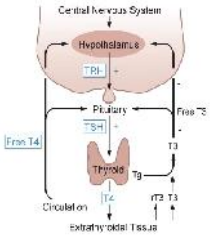


OBJECTIVE - 4

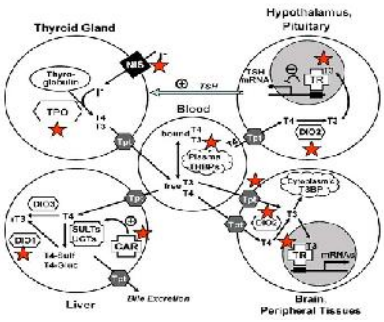
Anti-thyroidal action of TBTCl and its mechanism of action *in vitro* and *in vivo*

Brief Introduction

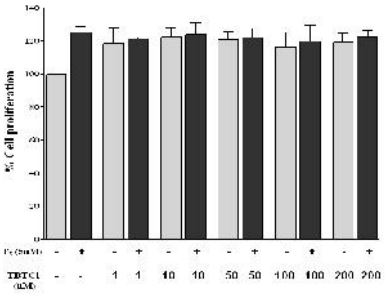
Thyroid function is regulated by an orchestrated mechanism of negative feedback among the circulating thyroid hormones at the hypothalamic–pituitary axis and works to maintain relatively stable serum levels of thyroid hormones within each individual.



Thyroid disruption Endpoints



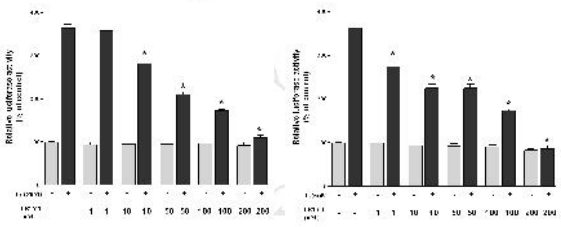
Cell proliferation assay- HepG2 cells



Sharan et al., 2014 *Gen Comp Endocrinol*. 206:155-65.

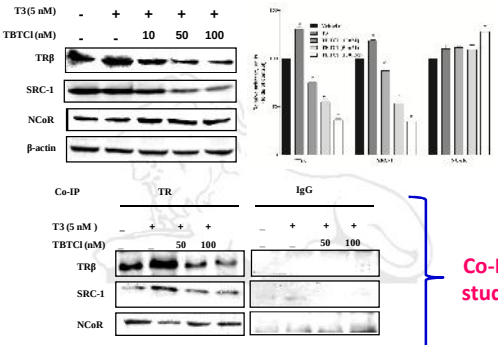
TR Transactivation assay in HepG2 cells

TRα Transactivation assay **TRβ Transactivation assay**



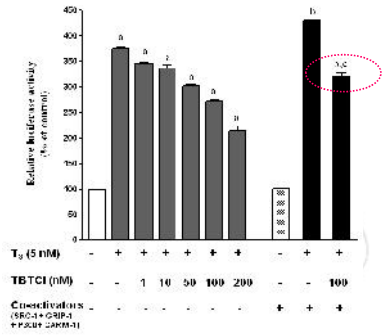
Sharan et al., 2014 *Gen Comp Endocrinol*. 206:155-65.

TBTCl alters the expression of TR and its co-regulators



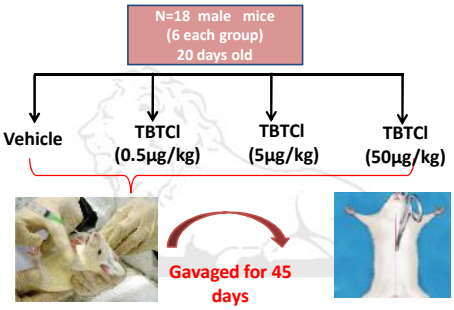
Sharan et al., 2014 *Gen Comp Endocrinol*. 206:155-65.

Deregulation of thyroid receptor expression is mediated through debarring co-activators and recruiting co-repressors



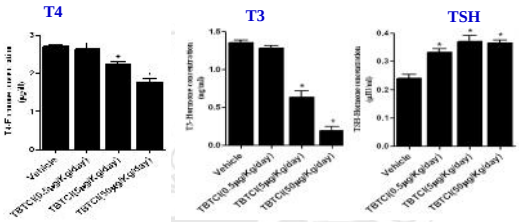
Sharan et al., 2014 *Gen Comp Endocrinol*. 206:155-65.

Animal Experimental design



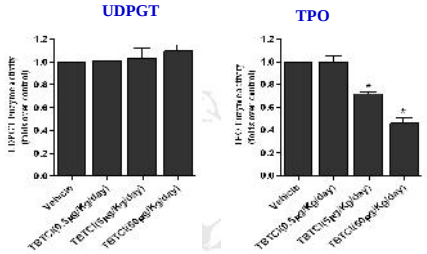
Mice Sacrificed at the end of trial and blood and organs were collected

Effect of TBTCl on the hormonal profiles of animals



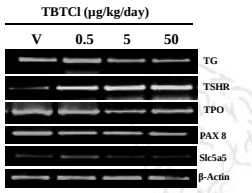
Sharan et al., 2014 *Gen Comp Endocrinol*. 206:155-65.

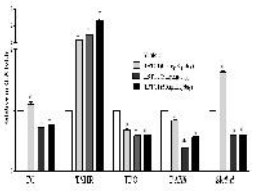
Effects of TBTCl on various thyroidal enzyme activities

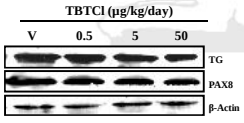


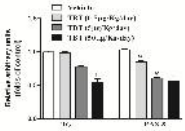
Sharan et al., 2014 *Gen Comp Endocrinol*. 206:155-65.

Gene and Protein expression of various thyroid related genes in thyroid gland of mice

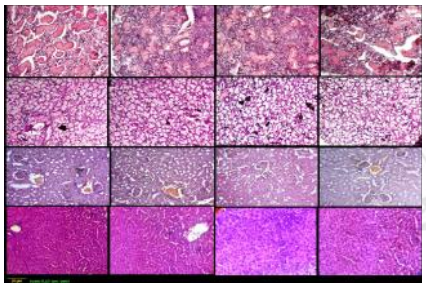








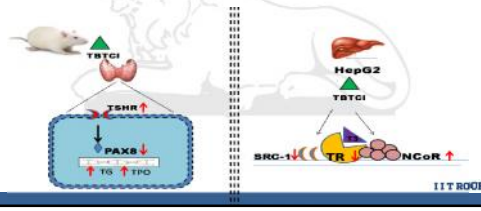
Histopathological analysis



Sharan et al., 2014 *Gen Comp Endocrinol*. 206:155-65.

CONCLUSION

- We have shown, for the first time that TBTCl is able to modulate the thyroidal axis.
- Low concentrations of TBTCl suppress TR transcription by disrupting the physiological concentrations of T3/T4, followed by the recruitment of NCoR to TR, providing a novel insight into the thyroid hormone-disrupting effects of this chemical.
- In the *in vivo* study, some of the genes linked to thyroid glands like TG, TPO, PAX8 etc were down- regulated in response to this chemical in mice.



OBJECTIVE - 5

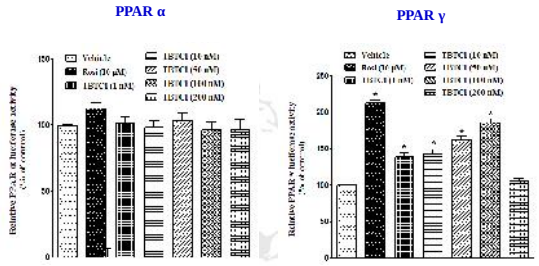
Understanding the obesogenic effect of TBTCl and its mechanism of action in vitro and in vivo

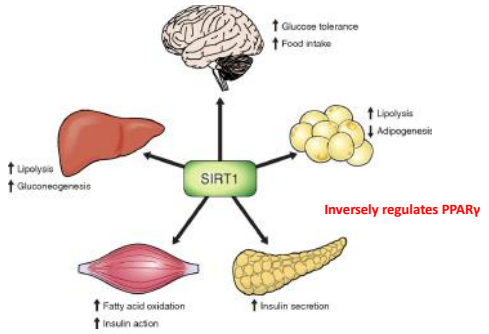
Brief Introduction

- Obesity is basically associated with the accumulation of excessive body fat, resulting from a chronic imbalance of energy whereby the intake of energy exceeds expenditure.
- The environmental factors that impact obesity has been referred to as "obesogens". TBTCl is a obesogen and is also a potent agonistic ligand of PPARγ [Grün and Blumberg, 2006]
- However, the exact mechanism of action in higher animal models are still lacking.
- The present study was undertaken to determine obesogenic effects of TBTCl using both *in vitro* and *in vivo* approaches.

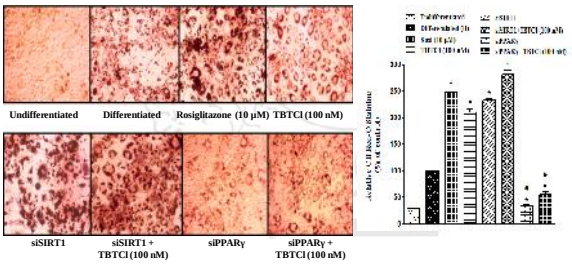
In vitro model – 3T3-L1, RAW- 264.7 cells
In vivo models - Swiss albino female mice

TBTCl alters PPAR γ transactivational activity in 3T3-L1 cells

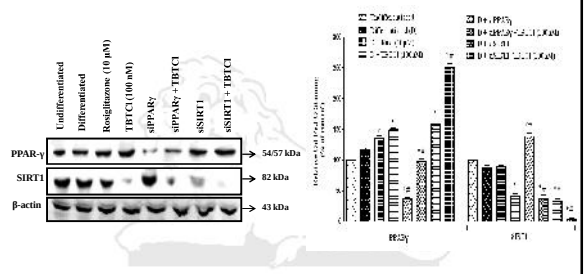




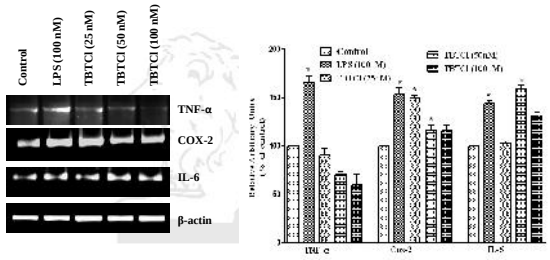
TBTCl promotes 3T3-L1 preadipocyte differentiation through up regulating PPARγ



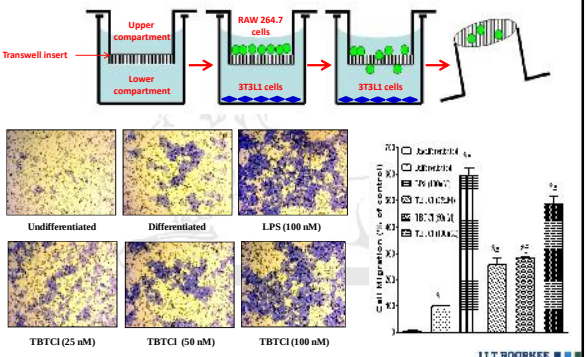
TBTCl promotes adipogenesis through PPARγ via SIRT1 inactivation



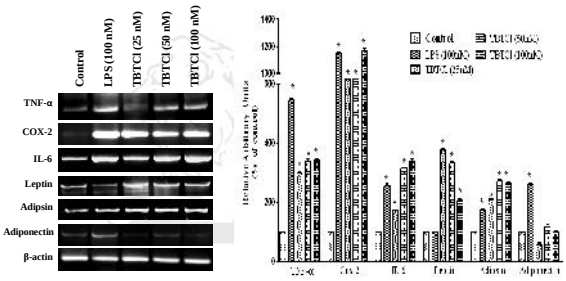
Effect of TBTCl on inflammatory molecules in 3T3-L1 cells



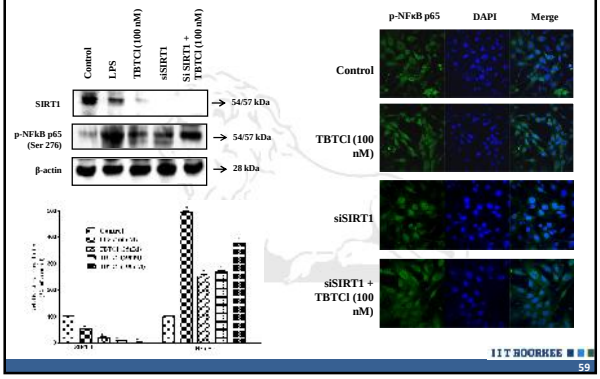
TBTCl potentiates recruitment of RAW264.7 macrophage to 3T3-L1 adipocyte



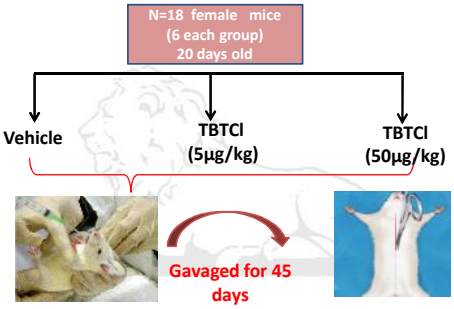
Effect of TBTCl on inflammatory marker expression in 3T3L1 adipocyte and RAW264.7 macrophage coculture



TBTCl promotes inflammation in adipose cell through NFκB via SIRT1 inactivation

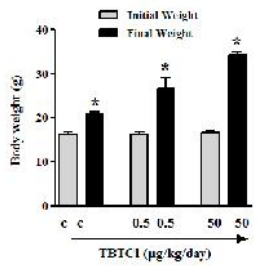


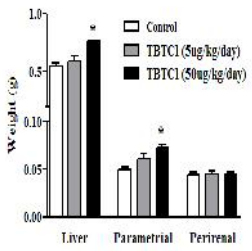
Animal Experimental design



Mice Sacrificed at the end of trial and blood and organs were collected

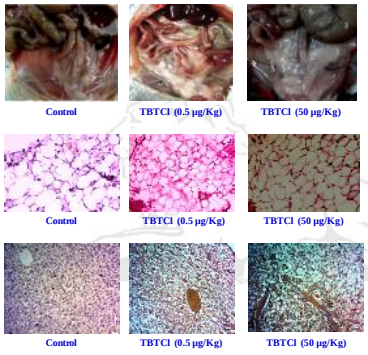
Exposure to TBTCl leads to induction of obesity in mice



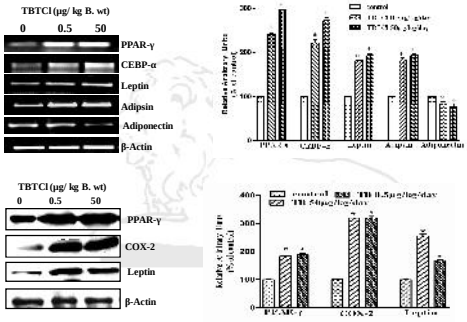


Body weight **Fat development**

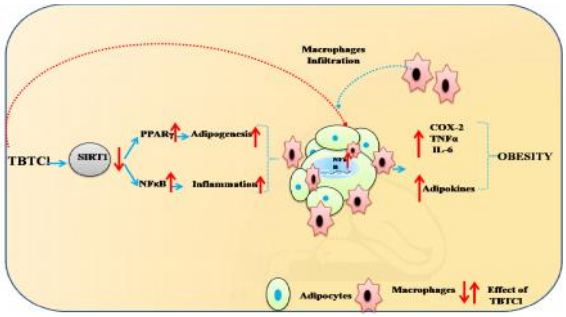
Exposure to TBTCl leads to induction of obesity in mice



TBTCl induces adipogenic regulators and markers *in vivo*



Conclusions



Summary

- **TBTCl was found estrogenic *in vitro* and *in vivo*.**
- **TBTCl was anti-thyroidal in both *in vitro* and *in vivo* models.**
- **Obesogenic potential of TBTCl *in vitro* and *in vivo* was through multiple mechanisms including increased adipogenesis and inflammation via SIRT1 inactivation.**

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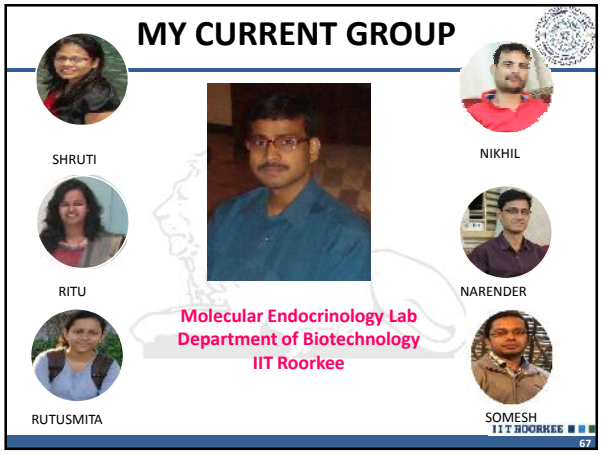
Indian Institute of Technology
Roorkee


MY CURRENT GROUP
SHRUTI
NIKHIL
RITU
NARENDER
RUTUSMITA
SOMESH
Molecular Endocrinology Lab
Department of Biotechnology
IIT Roorkee
IIT ROORKEE
67

Thank You. . .

Endocrine Disrupting Chemicals

An overview of health impacts and regulation

David Gunnarsson, PhD
Swedish Society for Nature Conservation

New Delhi
February 4, 2016

Swedish Society for Nature Conservation

- **Founded in 1909**
- **221 000 members –the largest environmental NGO in Sweden**
- **Politically and religously unbound organization**
- **Identify environmental threats**
- **Present innovative solutions to environmental problems**
- **Information to the public**

Endocrine disrupting chemicals (EDC)

Definition

"An endocrine disrupting chemical is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse effects in an intact organism, or its progeny, or (sub)populations."

International Programme on Chemical Safety (IPCS), WHO

Endocrine disrupting chemicals (EDC)

Presence

Industrial chemicals:
PCB:s, nonylphenol , phthalates, bisphenol A, brominated flame retardants

Pesticides:
DDT, linuron, vinclozolin

Cosmetics:
Propyl- and butylparaben, resorcinol

Pharmaceuticals:
DES, Finasteride, Trenbolone

Naturally occuring substances:
Metals (eg. cadmium), phytoestrogens

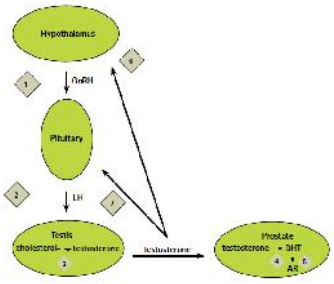
Endocrine disrupting chemicals (EDC)

Mechanisms of action

An EDC may disturb endocrine functions by interfering with

- Synthesis and/or
- Secretion and/or
- Signalling of a peptide or steroid hormone

EDC:s may target on or several steps in the HPG axis



Swedish Society for Nature Conservation 2011. Save the men

Grouping of EDC:s based on their mechanism(s) of action

Estrogenic	Genistein
	PCB:s
	Bisphenol A
	Endosulfan
	Zeranol
	Brominated flame retardants
	UV filters
Anti-estrogenic	PCB
	Prochloraz
Androgenic	Trenbolone
	TBECH
Anti-androgenic	Vinclozolin
	DDE
	Dioxin
	Phthalates
	Brominated flame retardants

Interferes with steroid synthesis	Prochloraz
	PFOA
	Parabens
	DBP
	DEHP
	Tributyltin
	Phytoestrogens

Mouritsen et al. 2010
Khalaf et al. 2009

Naturskyddsföreningen

Endocrine disrupting chemicals (EDC)

Effects on public health

EDC exposure have been associated with:

- Reproductive malformations and disorders
- Diabetes
- Obesity
- Cardiovascular disease
- Neuropsychological diagnoses

Further reading:

State of the Science of Endocrine Disrupting Chemicals – 2012, WHO/UNEP

Naturskyddsföreningen

Examples of EDC exposure associated with negative health effects

Bisphenol A exposure was positively associated with obesity in US children

Eng et al., Pediatrics 2013

Urinary PAH was associated with higher BMI and obesity in children

Scinicariello och Buser, Environ Health Persp. 2013

Boys that were exposed to PCB during fetal life had significantly lower semen quality at the age of 20 than other boys

Savage et al., J Allergy Clin Immunol, 2012

Boys that were exposed to DDT during fetal life had an increased risk of developing testicular cancer 30 years later

Cohn et al., 2010

Naturskyddsföreningen

Endocrine disrupting chemicals (EDC)
- Special characteristics

Non-monotonic dose-response relationships
The old motto "the dose makes the poison" is often untrue. A low dose may have the opposite effect compared to a high dose.

Timing of exposure is crucial
For example, some "windows" during fetal life may by extremely sensitive

Often a long time from exposure to effects, making it difficult to discover relationships

Certain population groups may be extra vulnerable
Genetic variations may increase/decrease the vulnerability significantly

The effects of exposure to an EDC is often dependent on the levels of endogenous hormones

Mixture toxicity
Low concentrations of individual EDC:s can together cause adverse effects (also substances from varying product groups)

Further reading:
Gunnarsson (2008). Reproductive Toxicology of endocrine disruptors. Doctoral Thesis

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Alarming trends in reproductive function!

More men get testicular cancer

	1973-1977	1998-2002	Change
	Incidence rate	*Incidence rate*	
Sweden	3,1	5,3	+ 70%
Finland	1,6	3,7	+ 131%

Incidence rate : New cases per 100.000 person years

Chia et al. 2010

Puberty starts earlier

	Girls	Boys
1991-93	10,9 years	11,9 years
2006-08	9,9 years	11,6 yeras

Askglaede et al. 2009
Sorensen et al. 2010

Decreasing sperm concentrations

	1989	2005
Sperm concentrations among french adult men (x 10^6/ml)	73,6	49,9

Rolland et al. 2013

Swedish Society for Nature Conservation 2011. Save the men

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Does EDC exposure contribute to the negative effects seen in the population?

Accumulating evidence:

Exposure to certain chemicals (in animal experiments) give rise to the same type of disorders as observed in the human population, eg. obesity, affected pubertal development and hypospadias

Associations between chemical exposure and these negative health effects have been found in epidemiological studies

Second-generation immigrants have the same frequency of these disorders as the inhabitants of the "new" country

Sharp increase in a short time period

Accidental releases of EDC:s has been found to affect reproductive function.

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Reducing EDC exposure reduces public expenditures

EU

The disease cost caused by EDC exposure has been estimated to approx. 153 billion Euros (corresponds to 1.23% of EU GDP)

Trasande et. al., J. Clin. Endocrinol. Metab. 2015

US

A ban on bisphenol A in food packaging is estimated to result in a cost reduction of 1.74 billion USD, as a consequence of less childhood obesity and cardiovascular disease.

Trasande, Health Aff. 2014

Africa

Exposure to lead among children is estimated to lower GDP by approximately 4%

Attina and Trasande, Environ. Health Persp. 2013

Sweden

Fractures caused by dietary cadmium exposure has been estimated to cost approximately 4 billion SEK (≈ 600 MUSD) per year

PM 12/12, Swedish Chemicals Agency. (In Swedish)

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EDC:s in a regulatory context

Biocides Regulation (EU) 528/2012

Chemicals legislation is almost totallly harmonized within the EU

Article 19

4. A biocidal product shall not be authorised for making available on the market for use by the general public where:

(d) it has endocrine-disrupting properties

Severely delayed by the EU Commission which decided to perform an impact assessment first

Article 5

3. No later than 13 December 2013, the Commission shall adopt delegated acts in accordance with Article 83 specifying scientific criteria for the determination of endocrine-disrupting properties.

During 2014, Sweden sued the EU Commission over the delay

December 2015: The general court finds that the EU Commission has breached EU law by not establishing the criteria

?

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SSNC´s opinions

Criteria for EDC:s, that are protective enough for human health and the environment, should be established ASAP.

Criteria should be set on a scientific basis only (i.e. impact assessment not relevant)

The use of substances listed as EDC:s by the International Chemical Secretariat (ChemSec) should be banned

National chemical restrictions should be allowed

Authorities should ensure that the legislation is followed

Introduce differentiated taxes on chemicals, making it profitable to use the least toxic ones

Public procurement should demand products free from EDC:s

Increase the use of scientific, non-GLP, studies in the risk assessment of chemicals
(Model for evaluation: Beronius et al. J Appl Toxicol. 2014 Jan 30)

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Thanks for your attention!

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Erosion of Cellular Transcription Memory by Endocrine Disrupting Chemicals

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New Delhi-110067

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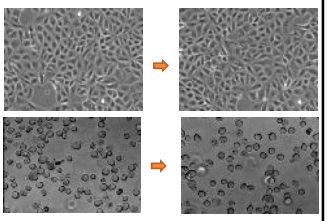
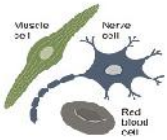
Cells can reproduce themselves continuously

- Self-renewal or self-replication is a normal biological process.
- When a hepatocyte divides it produces hepatocytes.
- When a fibroblast divides it reproduces fibroblasts.



Epigenetic Marks Define Cell Types

- Different cell types from an organism contain the same set of DNA/genome but different proteome.
- Epigenetic marks (methylation, acetylation etc.) are now known silence expression of certain gene while activate others.
- For normal functioning of the cell, these marks have to work in harmony.
- If the marks do not work properly the cell may begin to malfunction or be eliminated.



Epigenetic Marks Define Cell Types

- **There are now evidence to suggest that a cell may maintain its proteome, its phenotype or its traits with the involement of NRs as epigenetic marks.**

3

Nuclear Receptor superfamily

- **Nuclear Receptor (NR) super-family is a large group of ligand-modulated transcription factors with 48 members presently identified in human genome.**
- **These transcription factors are implicated in numerous physiological processes, and have been prospective drug targets for treatment of many endocrine-related diseases (e.g. breast/prostate cancers).**
- **Ligand-induced movements of transcription factors is one of the major phenomena in regulating their transcriptional activity and GFP-tagged NR chimera have been used to study their dynamic behavior in living cells.**
- **Discovery of GFP and availability of multi-coloured fluorescent variants, in conjunction with advances in fluorescence microscopy, have revealed finer details of protein localization and sub-cellular dynamics in living cells.**

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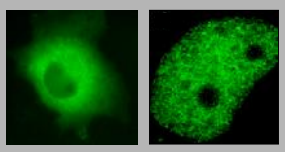
Sub-nuclear Distribution of GFP-tagged AR

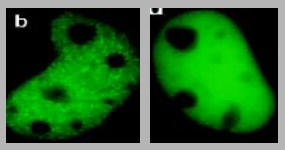
'Nuclear foci' correlate with transcriptional status of the receptor

Unliganded



DHT
(Natural ligand)
Transcriptionally active

Cyp Ac
(Partial agonist)

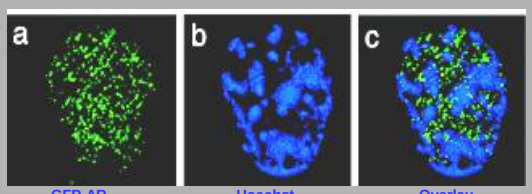


Casodex
(Pure antagonist)
Transcriptionally inactive

Tyagi RK *et al* **2000** Mol. Endocrinol.

6

A three-dimensional image analysis of the intranuclear localization of the androgen-bound AR-GFP

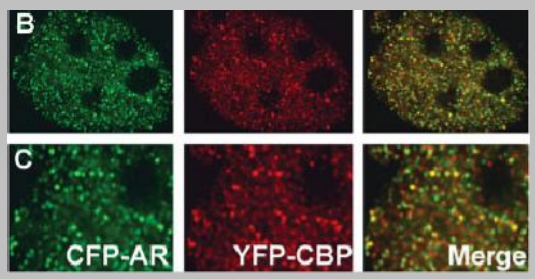


GFP-AR Hoechst Overlay

- In the single nucleus of the DHT-treated cells, about 250-400 bright GFP spots existed as a distinct volume.
- Most of the GFP spots were located in the peripheral zone of the euchromatin region adjacent to the heterochromatin region in which Hoechst 33342 staining was more dominant.

Tomura *et al* (2001) J Biol Chem.

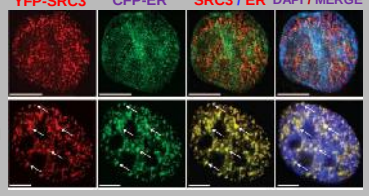
AR co-localizes with CBP in 'nuclear foci'



HeLa cells were co-transfected with **CFP-AR** (shown in green) and **YFP-CBP** (shown in red) and treated with DHT for 1 hour. C: The same cell is shown at higher magnification to show **regions of overlap between CFP-AR and YFP-CBP** more clearly. Marcelli M *et al* **2006** J. Cell. Biochem.

8

ERa co-localizes with coactivator SRC3 in 'nuclear foci'



Fluorescence microscopy was performed on cells expressing **CFP-ER** (pseudocolored in green) and **YFP–SRC-3** (pseudocolored in red). Forty-eight hours after transfection, cells were treated with 10 nM E2 for 2 h or with vehicle and counterstained with DAPI. A *Z*-series of focal planes was digitally imaged and deconvolved to generate high-resolution images. In the absence of hormone (top row), CFP-ER and YFP–SRC-3 were relatively diffused in the nucleus. The addition of ligand (bottom row) resulted in a **redistribution of both proteins into the same foci**, some of which are indicated by arrows . Amazit L *et al* 2007 MCB

So, it was apparent that 'nuclear foci' represents transcriptionally active receptor while homogeneously distributed receptor represents transcriptionally inactive receptor

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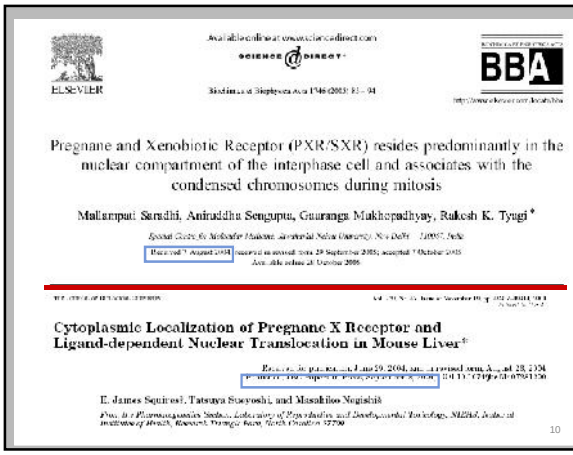

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BBA

Pregnane and Xenobiotic Receptor (PXR/SXR) resides predominantly in the nuclear compartment of the interphase cell and associates with the condensed chromosomes during mitosis

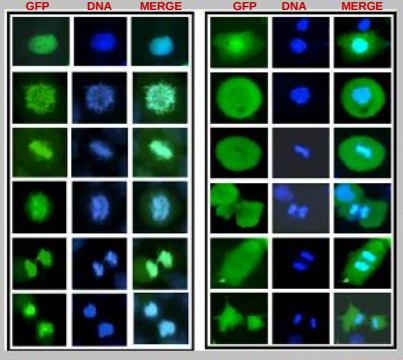
Mallampati Saradhi, Aniruddha Sengupta, Gaurangs Mukhopadhyay, Rakesh K. Tyagi *

Cytoplasmic Localization of Pregnane X Receptor and Ligand-dependent Nuclear Translocation in Mouse Liver*

E. James Squires†, Tatsuya Sueyoshi, and Masahiko Negishi§

10

PXR associates with condensed chromosomes during mitosis



Saradhi *et al* 2005 BBA Mol. Cell Res.

11

- We know that gene transcription is globally silenced during mitosis.
- Question: Why should a transcription factor associate with condensed chromatin during mitosis?

Speculations
- Role in chromatin condensation
- Preventing damaging events to genome
- Early onset of PXR-regulated genes

12

❖ Along with a couple of examples (TBP, HMGP), nuclear Receptor PXR, was the first transcription factor observed to be constitutively associated with the mitotic chromatin.

❖ So, our next query was to examine if other NRs also associate with the mitotic chromatin? Is it a general phenomenon?

❖ To extend our study further, we selected two more well-characterized transcription factors i.e. AR (cytoplasmic) and ERα (nuclear).

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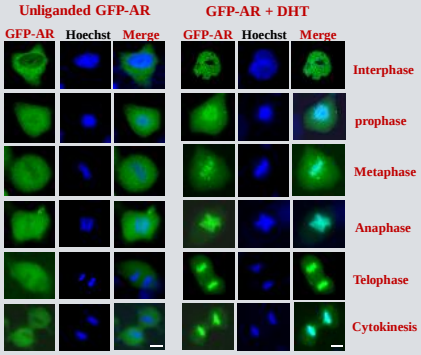
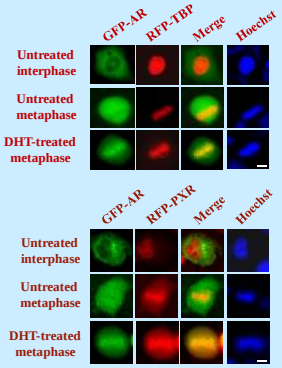
Androgen-mediated docking of GFP-AR onto mitotic chromatin



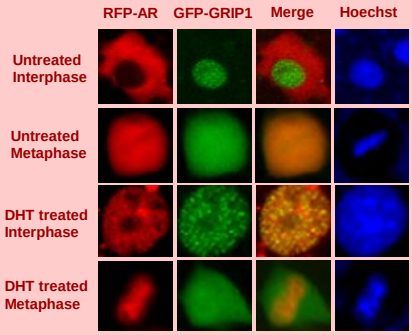
Kumar *et al*, **2008** BBA-Mol. Cell Res.

Ligand-activated AR binding onto the mitotic chromatin is neither impeded nor inhibited by co-expressed TBP or PXR



15

Co-activator GRIP-1 that co-localizes with androgen-activated AR in interphase is abrogated during mitosis



16

❖ It is well-established that interphase chromatin undergoes extreme compaction during mitosis (ratio 1:10,000) and active gene transcription is silenced.

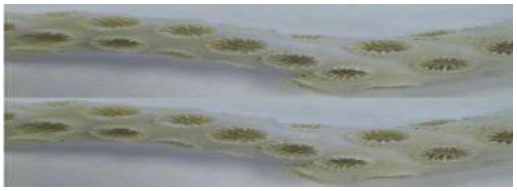
❖ If gene transcription is silenced during mitosis, the dynamic association of transcription factors with mitotic chromatin should have some physiological significance.

❖ Interestingly, there are now a few examples of proteins associating with mitotic chromatin and occupying target gene promoters that are maintained in an open chromatin configuration even during mitosis.

(eg. HMGN1, CTCF, FoxI1, cyclin B1).

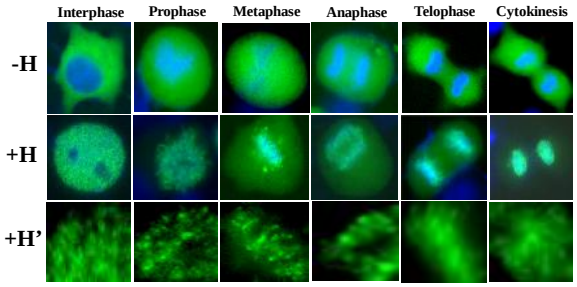
17

Close-up of calcium carbonate skeleton secreted by the coral



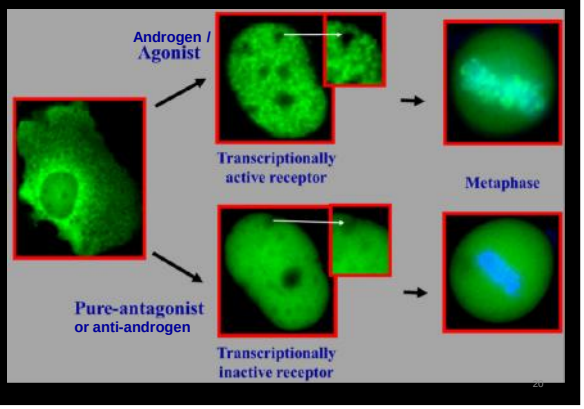
18

Inheritance of 'nuclear foci' from progenitor to progeny - a visual evidence from living cells



19

Androgen Receptor dynamics during interphase and mitosis in living cells



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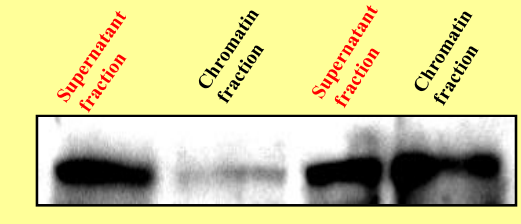
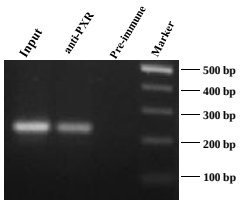
AR binding to mitotic chromatin is androgen-mediated



21

Nuclear receptor PXR binds to ER-6 sequences of its target promoters during mitosis



The ChIP primer sequence ER6 of the CYP3A4 promoter
ER6 F - 5'ATGCCAATGGCTCCACTTGAG 3'
ER6 R - 5'CTGGAGCTGCAGCCAGTAGCAG 3'

22

❖ From our observations we hypothesized that, in simple analogy to epigenetic markings, existence of 'biomolecular marking' by the ligand-activated transcription factors for transmitting the pattern of active gene transcription from interphase nucleus, via mitosis, to emerging daughter cells may also be operational in cells.

❖ This may be a natural process that cells utilize for ensuring the inheritance of progenitor's '*active gene transcripts*' to their progeny.

❖ Retention and feed-forward transmission of '*active gene transcripts*' should help in maintenance of inherent cellular characteristics and cell phenotype.

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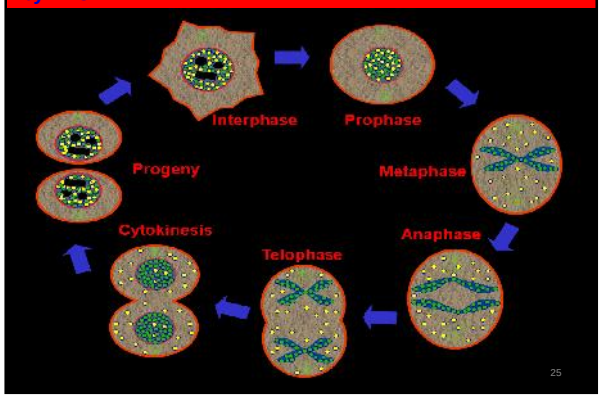
❖ Therefore, to distinguish from 'epigenetic markings' we have proposed a novel '*BIOPIT* concept' exemplifying '**B**iomolecular **I**mprints **O**ffered to **P**rogeny for **I**nheritance of **T**raits, *via* transcription factors (NRs) themselves.

❖ In this process the transcript factor itself serves as an epigenetic mark to transmit the transcription pattern to the next generation after exit from the mitosis.

Chaturvedi et al Endocrine disruptors provoke differential modulatory responses on Androgen Receptor and Pregnane & Xenobiotic Receptor: potential implications in metabolic disorders. ***Mol. Cell. Biochem.*** 345 : 291-308.

24

Retention and transmission of active transcription memory by NRs



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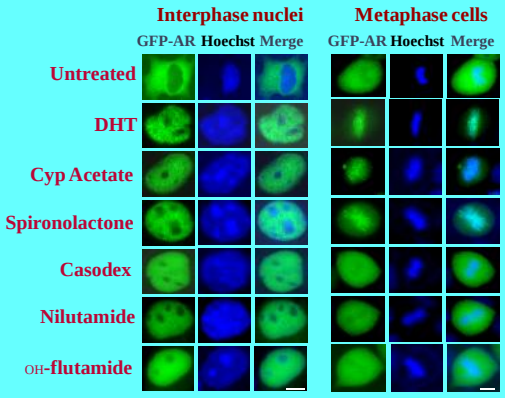
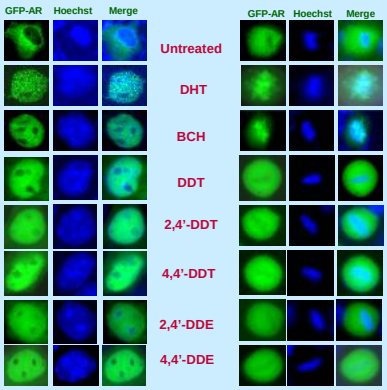
Effect of prostate cancer drugs on AR BIOPIT pattern



26

Effect of Endocrine Disruptors on AR BIOPIT pattern



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Consequence of molecular intervention on 'BIOPIT process'

❖Both AR and ERα are targeted by therapeutic anti-hormone drugs for treatment of hormone-responsive prostate/breast cancers.

❖ A number of endocrine disrupting chemicals which includes environmental/industrial chemicals are also known to act as antagonist/anti-hormone (includes many pesticides).

❖ We hypothesize that anti-hormone drugs / endocrine disruptors may erode the BIOPIT patterns of NRs resulting in loss of transmission of transcription memory from progenitor to progeny cell?

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❖ Therapeutic or chemical intervention may alter this natural process of retention and transmission cellular memory resulting in erosion loss of this natural inheritance process.

❖ Advanced stages of prostate/breast cancer may be good examples where disruption of this process by therapeutic intervention may be occurring. Consequently, this may impose upon cells to undertake alternative survival routes.

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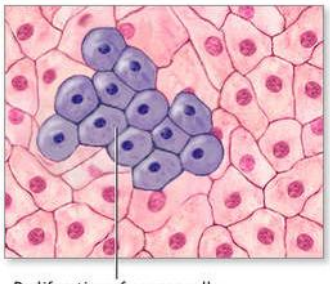

Proliferation of cancer cells

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SUMMARY

❖ It appears that cells utilize a process of retention and transmission of active transcription memory from progenitor to progeny via ligand-modulated transcription factors.

❖ Therapeutic or chemical intervention may alter this natural process of retention and transmission cellullar transcription memory.

❖ Nuclear Receptors that maintain the cell specific proteome may act as epigenetic marks. Disruption of these these marks may alter the proteome of the cells leading to apoptosis or may impose upon some cells to survive dysregulation leading to uncontrolled proliferation of cells (cancer)..

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RELEVENT PUBLICATIONS

Tyagi RK, Lavrovsky Y, Ahn CS, Song SC, Chatterjee B and Roy AK (2000) Dynamics of intracellular movement and nucleocytoplasmic recycling of the ligand-activated androgen receptor in living cells. ***Molecular Endocrinology*** 14 : 1162-1174 **(Journal's cover article).**

Saradhi M, Sengupta A, Mukhopadhyay G and Tyagi RK (2005) Pregnane and Xenobiotic Receptor (PXR) resides predominantly in the nuclear compartment of the interphase cell and associates with the condensed chromosomes during mitosis. ***Biochimica et Biophysica Acta - Molecular Cell Research*** 1746 : 85-94.

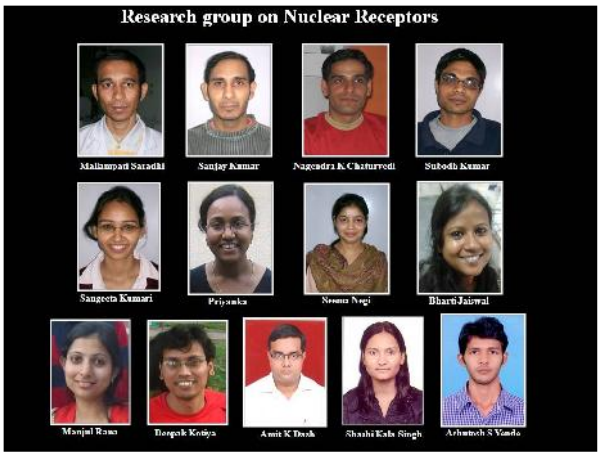
Kumar S, Chaturvedi NK, Kumar S and Tyagi RK (2008) Agonist-mediated docking of androgen receptor onto the mitotic chromatin platform discriminates intrinsic mode of action of prostate cancer drugs. ***Biochimica et Biophysica Acta -Molecular Cell Research*** 1783 : 59-73.

Chaturvedi NK, Kumar S, Negi S, Tyagi RK (2010) Endocrine disruptors provoke differential modulatory responses on Androgen Receptor and Pregnane & Xenobiotic Receptor: potential implications in metabolic disorders. ***Molecular and Cellular Biochemistry*** 345 : 291-308.

Kumar S, Saradhi M, Chaturvedi NK, Tyagi RK (2012) Retention and transmission of active transcription memory from progenitor to progeny cells via ligand-modulated transcription factors. ***Cell Biology International*** 36 : 177-182. **(Journal's highlight)**

Kumar S, Tyagi RK (2012) Androgen receptor association with mitotic chromatin: analysis with introduced deletions and disease-inflicting mutations. ***FEBS Journal*** 279: 4598-4614 **(Journal's cover article)**

33



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Agonist-mediated docking of androgen receptor onto the mitotic chromatin platform discriminates intrinsic mode of action of prostate cancer drugs

Sanjay Kumar, Nagendra K. Chaturvedi, Subodh Kumar, Rakesh K. Tyagi *
Special Centre for Molecular Medicine, Jawaharlal Nehru University, New Delhi 110067, India

Received 27 April 2007; received in revised form 2 October 2007; accepted 5 November 2007
Available online 19 November 2007

Abstract

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Keywords: Steroid receptor; Mitotic chromatin; Antiandrogen; Prostate cancer

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Mol Cell Biochem
DOI 10.1007/s11010-010-0583-6

Endocrine disruptors provoke differential modulatory responses on androgen receptor and pregnane and xenobiotic receptor: potential implications in metabolic disorders

Nagendra Kumar Chaturvedi · Sanjay Kumar · Seema Negi · Rakesh K. Tyagi

Received: 22 March 2010 / Accepted: 28 August 2010
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Abstract A systematic comparison of the impact of some potential endocrine disruptors (EDs) on modulation of androgen receptor (AR) and pregnane and xenobiotic receptor (PXR) function was conducted in a multi-step analysis. Promoter-reporter-based transcription assays were performed in conjunction with receptor dynamic studies in living cells that helped implicating the suspected EDs for their deleterious effects. We demonstrate that most of the selected EDs not only inhibit AR transcriptional activity, but also alter its subcellular dynamics. Conversely, some of these anti-androgenic compounds were potent activator of xeno-sensing nuclear receptor, PXR. Interestingly, agonist-activated AR that associates with the mitotic chromatin fails to achieve this association when bound to anti-androgenic EDs. Conclusively, most EDs (except BCH) behaved like pure antagonist for AR while as agonist for PXR. Subsequent experiments with DDT treatment in mice model indicated that in testis AR and its regulated genes PEM and ODC levels are down-regulated, whereas in liver of same mice PEM is up-regulated while AR and ODC remain unchanged. On the contrary, PXR and its regulated genes CYP3A11 and MDR1 levels in mice liver were up-regulated while in testis PXR remained unchanged, CYP3A11 up-regulated and MDR1 were down-regulated. Based on a novel "Biopit" concept it is speculated that long-term exposure to endocrine disrupting chemicals may influence the epigenetic profile of target cells via transcription factors thereby making them vulnerable to onset of chemically induced endocrine-related malignancies or metabolic disorders.

Keywords Androgen receptor · Pregnane and xenobiotic receptor · Endocrine disruptors · Mitotic chromatin · Green fluorescent protein

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Determinants of Androgen receptor association with mitotic chromatin: analysis with introduced deletions and disease-inflicting mutations.
Kumar S and Tyagi RK (2012) 279:4598-4614

Now we have identified a **M**itotic **C**hromatin **B**inding-determining **R**egion **(MCBR)** within Androgen receptor.

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Cell
Biology
International

Volume 36, part 2 (February 2012)

Issue highlight

Retention and transmission of active transcription memory from progenitor to progeny cells via ligand-modulated transcription factors: elucidation of a concept by BIOPIT model
Sanjay Kumar, Mallampati Saradhi, Nagendra Kumar Chaturvedi and Rakesh Kumar Tyagi
Kumar et al. explore the BIOPIT model, and attempt to explain how disruption of BIOPIT markings by therapeutic anti-hormones or endocrine disruptors over prolonged periods may lead to eradication of cellular transcription memory with deleterious cellular consequences.

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Model explains the emergence of new genre of cells with deviant memory leading to advanced stages of prostate & breast cancers.

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PERSPECTIVES

OPINION

Mitotic bookmarking of genes: a novel dimension to epigenetic control

Sayyed K. Zaidi, Daniel W. Young, Martin A. Montecino, Jane B. Lian, Andre J. van Wijnen, Janet L. Stein and Gary S. Stein

Abstract | Regulatory machinery is focally organized in the interphase nucleus. The information contained in these focal nuclear microenvironments must be inherited during cell division to sustain physiologically responsive gene expression in progeny cells. Recent results suggest that focal mitotic retention of phenotypic transcription factors at promoters together with histone modifications and DNA methylation — a mechanism collectively known as gene bookmarking — is a novel parameter of inherited epigenetic control that sustains cellular identity after mitosis. The epigenetic signatures imposed by bookmarking poise genes for activation or suppression following mitosis. We discuss the implications of phenotypic transcription factor retention on mitotic chromosomes in biological control and disease.

NATURE REVIEWS | GENETICS VOLUME 11 | AUGUST 2010 | 585

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MOLECULAR AND CELLULAR BIOLOGY, Oct. 2010, p. 4758–4766 Vol. 30, No. 20
0270-7306/10/$12.00 doi:10.1128/MCB.00646-10
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MINIREVIEW

Architectural Epigenetics: Mitotic Retention of Mammalian Transcriptional Regulatory Information

Sayyed K. Zaidi,[1] Daniel W. Young,[1]† Martin Montecino,[2] Jane B. Lian,[1] Janet L. Stein,[1] Andre J. van Wijnen,[1] and Gary S. Stein[1]*
Department of Cell Biology and Cancer Center, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, Massachusetts 01655,[1] and Centro de Investigaciones Biomedicas, Facultad de Ciencias Biologicas y Facultad de Medicina, Universidad Andres Bello, Santiago, Chile[2]

Epigenetic regulatory information must be retained during mammalian cell division to sustain phenotype-specific and physiologically responsive gene expression in the progeny cells. Histone modifications, DNA methylation, and RNA-mediated silencing are well-defined epigenetic mechanisms that control the cellular phenotype by regulating gene expression. Recent results suggest that the mitotic retention of nuclease hypersensitivity, selective histone marks, as well as the lineage-specific transcription factor occupancy of promoter elements contribute to the epigenetic control of sustained cellular identity in progeny cells. We propose that these mitotic epigenetic signatures collectively constitute architectural epigenetics, a novel and essential mechanism that conveys regulatory information to sustain the control of phenotype and proliferation in progeny cells by bookmarking genes for activation or suppression.

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MOLECULAR AND CELLULAR BIOLOGY, Feb. 2011, p. [illegible]
0270-7306/11/$12.00 doi:10.1128/MCB.00710-10
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Vol. 31, No. 4

Genomic Profiling of HMGN1 Reveals an Association with Chromatin at Regulatory Regions

Suresh Cuddapah,[1] Dustin E. Schones,[1] Kairong Cui,[1] Tae-Young Roh,[1] Artem Barski,[1] Gang Wei,[1] Mark Rochman,[2] Michael Bustin,[2] and Keji Zhao[1]*
Laboratory of Molecular Immunology, National Heart, Lung and Blood Institute,[1] and Protein Section, Laboratory of Metabolism, National Cancer Institute,[2] National Institutes of Health, Bethesda, Maryland 20892

Received 15 June 2010/Returned for modification 27 July 2010/Accepted 9 December 2010

The interaction of architectural proteins such as the linker histone H1 and high-mobility-group (HMG) proteins with nucleosomes leads to changes in chromatin structure and histone modifications and alters the cellular transcription profile. The interaction of HMG proteins with chromatin is dynamic. However, it is not clear whether the proteins are constantly and randomly redistributed among all the nucleosomes or whether they preferentially associate with, and turn over at, specific regions in chromatin. To address this question, we examined the genome-wide distribution of the nucleosome binding protein HMGN1 and compared it to that of regulatory chromatin marks. We find that HMGN1 is not randomly distributed throughout the genome. Instead, the protein preferentially localizes to DNase I hypersensitive (HS) sites, promoters, functional enhancers, and transcription factor binding sites. Our results suggest that HMGN1 is part of the cellular machinery that maintains transcriptional fidelity by generating, maintaining, or preferentially interacting with specific sites in chromatin.

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Review

Cancer Research

Bookmarking Target Genes in Mitosis: A Shared Epigenetic Trait of Phenotypic Transcription Factors and Oncogenes?

Sayyad K. Zaidi[1,2], Rodrigo A. Grandy[1,2], Cesar Lopez-Camacho[1,2], Martin Montecino[3,4], Andre J. van Wijnen[5,6], Jane B. Lian[1,2], Janet L. Stein[1,2], and Gary S. Stein[1,2]

Abstract

The regulatory information for phenotype, proliferation, and growth of normal and tumor cells must be maintained through genome replication in the S phase and cell division during mitosis. Epigenetic mechanisms that include DNA methylation, posttranslational modifications of histones, selective utilization of histone variants, and inheritable RNA molecules play pivotal roles in maintaining cellular identity through mitotic divisions. Recent studies demonstrate that mitotic occupancy of genes, which are determinants of cell fate, growth, and proliferation, by lineage-restricted transcription factors is a key epigenetic mechanism for retention and transmission of cellular expression memory. Evidence is emerging for the presence of distinct transcriptional regulatory microenvironments in mitotic chromosomes in which the genes bookmarked for reactivation postmitotically reside. Importantly, some oncoproteins are present in mitotic microenvironments where they occupy target genes during mitosis and may contribute to perpetuating the transformed phenotype. We discuss emerging regulatory implications of epigenetically bookmarking genes during mitosis for physiologic control as well as for the onset and progression of cancer. Cancer Res; 74(2); 420–5. ©2014 AACR.

Cancer Res; 74(2) January 15, 2014

AACR American Association for Cancer Research

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the FEBS Journal

Androgen receptor association with mitotic chromatin – analysis with introduced deletions and disease-inflicting mutations

Sanjay Kumar and Rakesh K. Tyagi
Special Centre for Molecular Medicine, Jawaharlal Nehru University, New Delhi, India

Keywords
androgen-insensitivity syndrome; nuclear localization signal; nuclear receptor; prostate cancer; transcription memory

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doi:10.1111/febs.12046

Sequence and structural anomalies in the gene for androgen receptor (AR) or its protein are associated with a range of clinical manifestations. Observations from living cells have shown that AR translocates to the nucleus upon ligand binding, where it forms typical 'nuclear foci' that are considered as sites of gene transcription. Recently, we reported the ligand-mediated association of AR with mitotic chromatin and suggested its role in 'transcription memory', proposing a 'biopit model'. In the present study, we show that each of the AR domains is obligatory for its association with mitotic chromatin and also that full-length AR is necessary for efficient association. In addition, deletion or point mutations in bipartite nuclear localization signal (NLS) revealed impaired localization, 'nuclear foci' formation and abolished AR binding with mitotic chromatin. Interestingly, well-characterized AR-NLS mutants associated with the manifestation of pathological conditions (prostate cancer and androgen-insensitivity syndrome) exhibited differential behaviour on mitotic chromatin and also impaired receptor localization and 'nuclear foci' formation. Finally, we report that, in addition to its functions in nuclear import, DNA binding, acetylation, N/C-termini interactions and transactivation, the AR-NLS region also functions as 'mitotic chromatin binding-determining region' and has a novel role in the regulation of the AR association with mitotic chromatin.

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The Times of India -Breaking news

Anti-cancer treatment may backfire

18 Nov 2007, 1307 hrs IST,PTI

NEW DELHI: Anti-cancer treatments may have an opposite effect on the cancerous cells increasing the small population of cancer stem cells believed to drive the disease, according to a latest research.

The research was undertaken by Vasyl Vasko, along with scientists from the CRTRC Institute for Drug Development in San Antonio and Johns Hopkins University after questions were raised on efficacy of some therapies used in treating cancer.

"Our experiments suggest that some treatments could be producing more cancer stem cells that then are capable of metastasizing, because **these cells are trying to find a way to survive the therapy**," said Vasko.

Vasko and his team measured both stem cells markers and tumor volume before and after treatment on mouse.

The stem cell markers contribute to stem cells' defining ability to **renew themselves and differentiate into different cell types**.

The researchers selected a rare form of cancer, Mesenchymal Chondrosarcoma (MCS), to study the expression of stem cell markers in metastasizing tumors and primary tumors.

They applied various therapies in mice implanted with human MSC, and analysed the effects on tumors.

Analysis of stem cell expression before and after treatment revealed that even as some anti-cancer treatments shrank tumors, they increased expression of stem cell markers. 46

THE TIMES OF INDIA, NEW DELHI
TUESDAY, JANUARY 6, 2009 17

Cancer cells can outwit chemotherapy

London: It is believed that chemotherapy uses chemicals to kill all cancer cells. But a new study says that some affected cells are capable to deceive the treatment.

Researchers have found that some cancers can "recover" from doses of chemotherapy which would destroy other types of cell — in fact, the diseased cells have a Houdini-like escape tactic which can outwit even potent drugs.

According to them, though it is believed that no cells could live after chemotherapy which triggers a process, called "apoptosis", or cell suicide, the study could explain why some cancers recur even after treatment.

Lead researcher Ming-Chiu Fung of Chinese University of Hong Kong was quoted by the Daily Telegraph as saying: "We have shown various cancer cell lines can survive programmed cell death. This research suggests existence of an escape tactic that cancer cells might call upon to survive chemotherapy. Our finding sparks new leads to research what drives cancer cells to come back to life after chemotherapy treatment."

In their study, the researchers subjected the cancer cells to three different types of the therapy, all of which cause apoptosis. In the laboratory, they gave the cells enough of the deadly chemicals to spark the impulse to die.

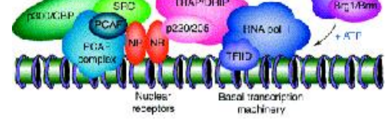
But they found that although the cells were damaged by the chemotherapy, once it was stopped they regained their original shape and continued to divide, causing them to grow and spread. The cells were mortally damaged only if their central part had already begun to disintegrate. However, this does not happen until right at the very end of the normal cell suicide process. PTI

NRI expert creates brain cancer model

Researchers led by an Indian American have developed a versatile mouse model of glioblastoma — the commonest of deadly brain cancers in humans. "Mouse models of human cancer have taught us a great deal about the basic principles of cancer biology," said Inder Verma, professor in the Laboratory of Genetics at the Salk Institute. "By definition, however, they are just that: approximations that simulate a disease but never fully capture the molecular complexity underlying disease in humans." IANS

CANCER WATCH

47



Multiple physical and functional interactions among nuclear receptors, coactivators, chromatin remodelers and chromatin lead to an ordered sequence of events culminating in the transcription of hormone-regulated genes including: (1) ligand-dependent interaction of coactivators with chromatin-bound NRs, (2) ATP-dependent chromatin remodeling by chromatin remodeling complexes, (3) histone acetylation by HAT coactivators and (4) contacts between NRs and the basal transcriptional machinery by the mediator-like coactivators (e.g. TRAP/DRIP). Whether functional interactions occur between the SRC-p300/CBP-PCAF coactivators and the TRAP/DRIP complex is currently unclear, as is indicated by the question mark.

Abbreviations: acetyl CoA, acetyl coenzyme A (the acetyl donor for acetylation reactions); CBP, CREB-binding protein; CREB, cAMP-response element-binding protein; HAT, histone acetyltransferase; NR, nuclear receptor; PCAF, p300/CBP-associated factor; p220/205, TRAP220/DRIP205; RNA pol II, RNA polymerase II; SRC, steroid receptor coactivator; TFIID, transcription factor IID [which contains TATA-binding protein (TBP) and TBP-associated factors]; TRAP/DRIP, thyroid hormone receptor-associated proteins/vitamin D receptor-interacting proteins.

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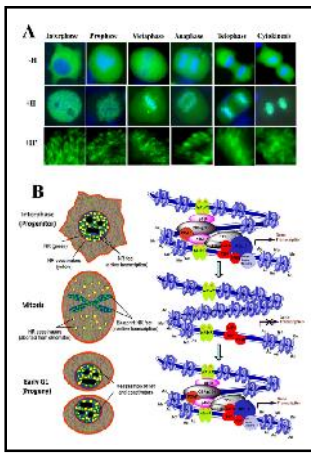

Biomolecular Imprints offered to Progeny for Inheritance of Traits (BIOPIT)

Epigenetic marks versus BIOPIT marks

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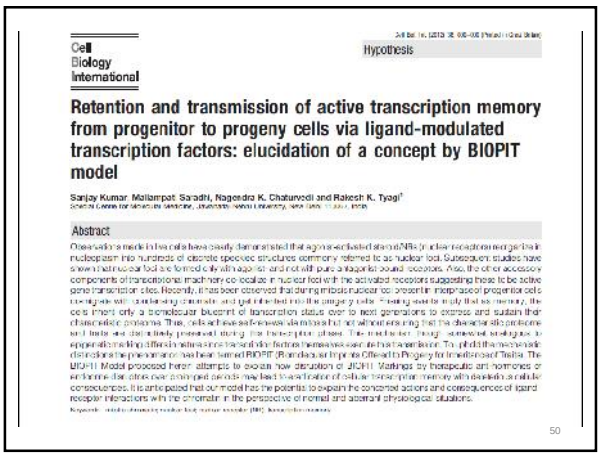

Cell Biology International

Hypothesis

Retention and transmission of active transcription memory from progenitor to progeny cells via ligand-modulated transcription factors: elucidation of a concept by BIOPIT model

Sanjay Kumar, Mallampat Saradhi, Nagendra K. Chaturvedi and Rakesh K. Tyagi

Abstract

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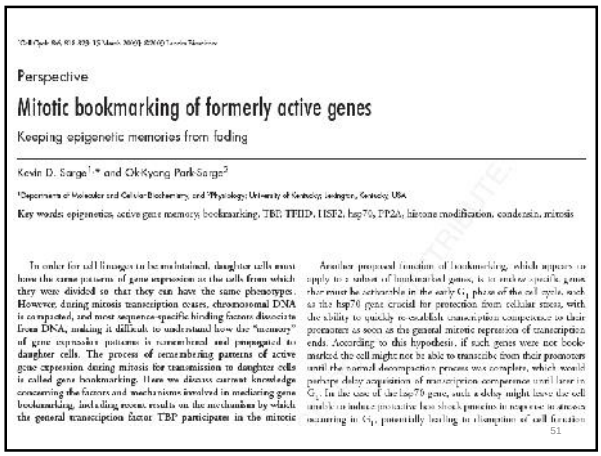


Perspective

Mitotic bookmarking of formerly active genes

Keeping epigenetic memories from fading

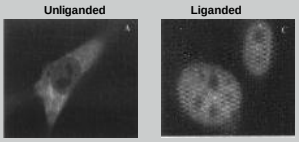
Kevin D. Sarge and Ok-Kyong Park-Sarge

Key words: epigenetics, active gene memory, bookmarking, TBP, TFIID, HSF2, hsp70, PP2A, histone modification, condensin, mitosis

51

Intracellular Distribution of GFP-hGRa and YFP-GRa

All ligand-bound nuclear receptors are nuclear. However, unliganded receptors may be either cyoplasmic of nuclear



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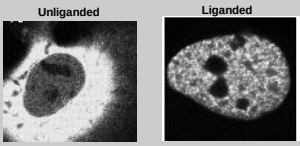

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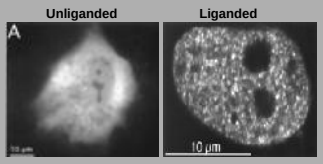
52

Intracellular Distribution of GFP-tagged MR and PR-B



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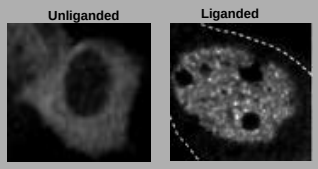

Lim CS *et al* **1999** Mol. Endocrinol.

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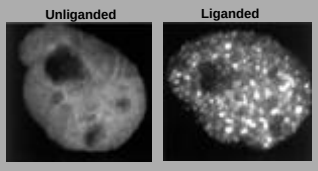
Intracellular Distribution of GFP/YFP-tagged ERa and RXR



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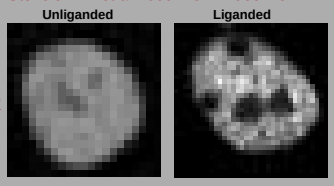

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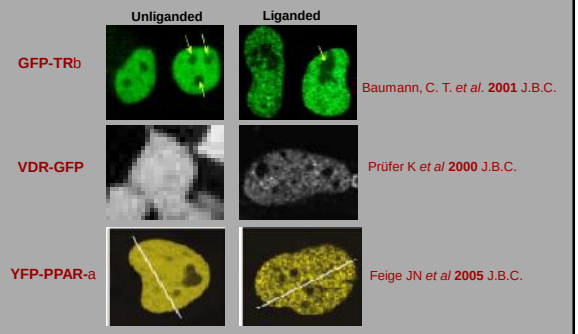
54

Intracellular Distribution of fluorescent protein-tagged TRb, VDR and PPAR-a



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Prüfer K *et al* **2000** J.B.C.

Feige JN *et al* **2005** J.B.C.

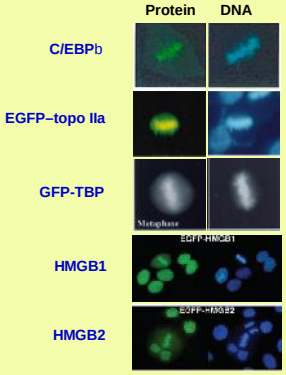
55

What is the mystery of these 'nuclear foci'?

56

Examples of some of the proteins associating with mitotic chromatin

(with a role in transcription regulation)



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Genes and Development

Mo and Beck **1999**
Expt. Cell Res.

Chen D *et al* **2002**
Mol. Biol. Cell

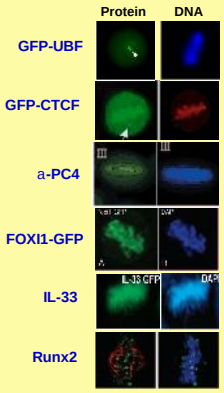
Pallier C **2003**
Mol. Biol. Cell

Pallier C **2003**
Mol. Biol. Cell

57

Subsequent examples of proteins associating with mitotic chromatin

(with a role in transcription regulation)



Chen D *et al* **2005**
J Cell Biol.

Burke L J *et al* **2005**
EMBO J

Das C *et al* **2006**
Mol. Cell Biol.

Yan J *et al* **2006**
Mol. Biol. Cell

Carriere V *et al* **2006**
PNAS

Young DW *et al* **2007**
PNAS

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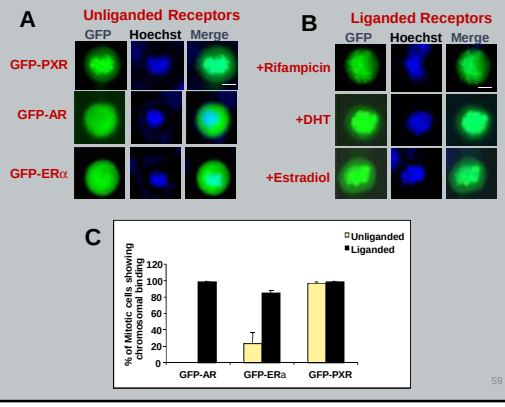
Association of steroid / nuclear receptors with mitotic chromatin



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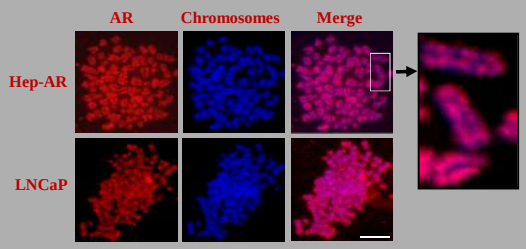
Chromosomal spreads reveal a higher order distribution of androgen-activated AR on chromosomes



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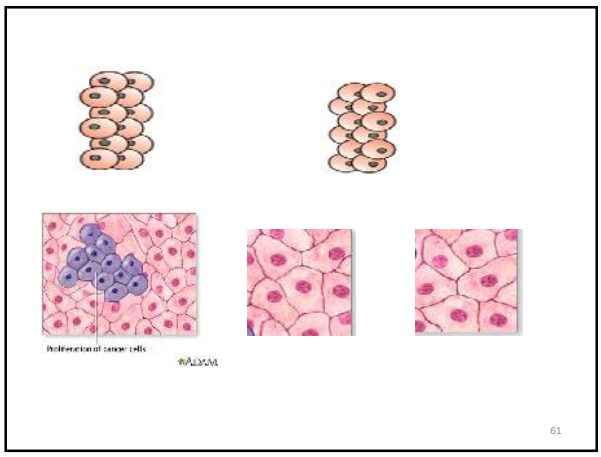
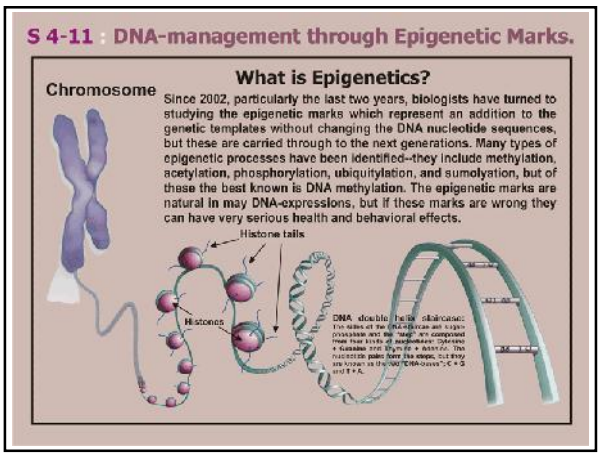
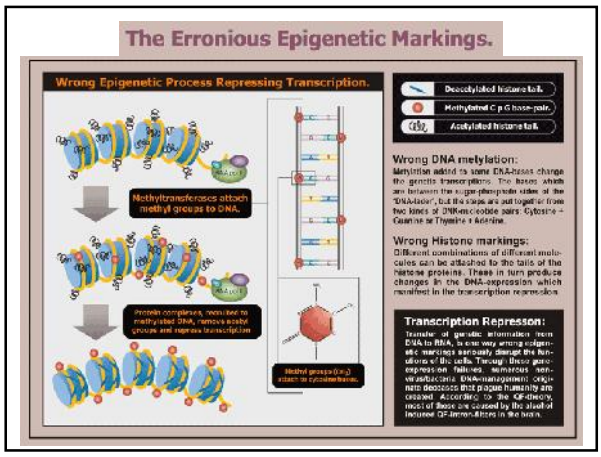

Proliferation of cancer cells
61


S 4-11 : DNA-management through Epigenetic Marks.
Chromosome
What is Epigenetics?
Since 2002, particularly the last two years, biologists have turned to studying the epigenetic marks which represent an addition to the genetic templates without changing the DNA nucleotide sequences, but these are carried through to the next generations. Many types of epigenetic processes have been identified--they include methylation, acetylation, phosphorylation, ubiquitylation, and sumolyation, but of these the best known is DNA methylation. The epigenetic marks are natural in may DNA-expressions, but if these marks are wrong they can have very serious health and behavioral effects.
Histone tails
Histones


The Erronious Epigenetic Markings.
Wrong Epigenetic Process Repressing Transcription.
Methyltransferases attach methyl groups to DNA.
Deacetylated histone tails
Methylated C p G bases-pair.
Acetylated histone tails
Wrong DNA metylation:
Wrong Histone markings:
Transcription Represson:

Impacts of Pharmaceutical: Effect of Sildenafil Citrate (Pink Viagra) on Physiological Functions in Female Rats

Manekar A. P.

Molecular Endocrinology Laboratory,
Department of Zoology, Institute of Science, 15, Madam Cama Road, Mumbai- 400 032, India
E mail: ashok.manekar@rediffmail.com

Infertility is one element of a spectrum of reproductive disorders that include miscarriage, congenital abnormalities, premature delivery and stillbirth and other physiological abnormalities.

- It is also defined as the failure to conceive, affecting about 50% of all couples at some time during their reproductive lives.
- Cause of infertility in women includes failure of ovulation, tubular damage, endometriosis and hostile cervical mucus, and hormonal imbalance etc.
- Drugs and toxins may be responsible for infertility but in general, effects of drugs on fertility and Sterility have been poorly studied in this aspect.
- The issue of sexual health once regarded as taboo subjects has been widely debated recently and provoked by production. Sildenafil, the first licensed oral treatment for male and female reproductive dysfunction has been marketed through out the world.

Our Research Team
Dr. Pragati Narase
Dr. Rajeshree Kakade
Dr. Varsha Narvade
Mr. Vishal Kadu
Miss. Shreya Patil

Bisphenol-A

Toxics Link, New Delhi

4th February 2016

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About Toxics Link

Toxics Link is an information, outreach and environmental advocacy organization set up in 1996

Programme Areas

Waste
- Biomedical waste,
- Electronic waste,
- Hazardous waste
- Municipal waste

Chemicals Safety
- Chemicals in Products,
- POPs and Pesticide,
- Mercury campaign
- Lead in Paint Campaign

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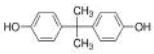
Bisphenol-A History

- Bisphenol – A is an **organic molecule** First synthesized in 1891
- It is produce by the condensation reaction between *acetone* and *phenol*, catalyzed by acid or a resin
- In 1950's, BPA was used to synthesize *polycarbonate plastics* and *epoxy resins*
- Before its known use as a chemical that makes plastic (1950), it was discovered as a **synthetic estrogen** in 1930's
- 6 billion pounds estimated use per year – used in consumer products



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Bisphenol-A Market

- $14.6 billion estimated globally in 2014, expected to reach to $21.9 billion till year 2022
- During forecast period 2014 to 2022 - Compound Annual Growth Rate (CAGR) of 5.2%
- Global geography is segmented into: North America, Europe, Asia-Pacific and Rest of the World.

BPA used so much because……

- it is highly **durable**
- It has a high **heat-resistance,**
- It is **break resistance** and
- It has high **optical clarity**

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Two kinds of BPA based plastics are available in the market

1. Polycarbonate (PC) plastics

- **Polycarbonates** are a group of thermoplastic polymers containing carbonate groups in their chemical structures
- Products: Electronics ; safety equipment ; automobiles ; food containers ;plastics with recycling code # 7 ; intravenous tubing ; dental sealant ; reusable water botles and baby bottles

2. Epoxy Resins

- **Epoxy Resins m**ade from a class of synthetic thermosetting polymers containing epoxide groups
- Products: Protective coatings of medical equipment ; piping ; steel drums, and interior of food/beverage/liquid infant formula cans

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Controversy !!

- The plastic industry is stating that the amount of BPA that a normal person is exposed to is **not toxic**

But……

- Other scientists and researchers have found that it causes many **health problems**

This resin code on a plastic container indicates that BPA may have been used in its manufacture.

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Health Problems

- Effects on the endocrine system occur at **very low doses**
- **An Endocrine Disrupter** - which means it mimics or interferes with body's hormones and "disrupts" endocrine system
- BPA has been linked to a number of health concerns, particularly in pregnant women, fetuses and young children, but also in adults, including:
  - Structural **damage** to the **brain**
  - Changes in gender-specific behavior, and **abnormal sexual behavior**
  - Increase **fat formation** and **risk of obesity**
  - Stimulation of prostate **cancer cells**
  - Hyperactivity, increase aggressiveness, and impaired learning

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Leaching BPA

- Monomers of BPA can hydrolyze and
- It leaches into food or liquid
- Accelerated by: High temperatures / Acidic or basic conditions / Damaged surfaces
- ***Shrinithivihahshini et al.*** (India), explained that BPA migrates from PC baby feeding bottles at on average 19ng/ml to hot water at 70^0C

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Toxics Link Studies on BPA in Children Products

Objectives

- To detect the presence of BPA in **BABY FEEDING BOTTLES** and **SIPPERS** sold in Indian market
- To find the claim of **BPA free**
- To highlight the urgent need of regulation for BPA in baby products
- To understand the overall consumer perspectives towards BPA and BPA free bottles in India

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Sample Details

- Baby Feeding Bottles – 14 nos.
  - **Most of the samples collected have labeled polycarbonate**
  - **4 samples were labeled as BPA free and polypropylene and**
  - **One of these four samples collected was labeled with "0% BPA"**
- Sipper Bottles – 13 nos.
  - **6 samples have been labeled as durable, safe, non toxics, 0% BPA or Safe for baby**

- **Lab Protocol - American Society for Testing & Materials (ASTM)-D7065-11; GC-MS**

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Results

Baby Feeding Bottles

- **78.5% samples contain BPA**
- **The maximum concentration – 9.4 ppm**
- **In 50% of BPA-free samples BPA had been detected above EU threshold limit of 0.6 ppm**
- **IS 14625 - The draft notification has stated that BPA will not be used in the baby feeding bottles**

Red marked samples were marked with BPA free

Samples	Concentration of BPA (ppm)
TLS - 1	0.1
TLS - 2	3.8
TLS - 3	0.1
TLS - 4	9.4
TLS - 5	0.1
TLS - 6	0.98
TLS - 7	0.88
TLS - 8	0.7
TLS - 9	0.22
TLS - 10	2.44
TLS - 11	0.86
TLS - 12	0.54
TLS - 13	0.44
TLS - 4	2.98

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Results

Sippy Cups

- **77% samples contain BPA**
- **The maximum concentration – 14.9 ppm**
- **In 83% (5/6) of BPA-free samples detected BPA**

FDA (2012) has amended its regulations to no longer provide for the use of BPA-based polycarbonate resins in baby bottles and sippy cups

Red marked samples were marked with BPA free

Samples	Concentration of BPA (ppm)
TL - 02	Not Detected
TL - 03	Not Detected
TL - 04	Not Detected
TL - 05	0.3
TL - 22	1.3
TL - 23	14.9
TL - 24	0.05
TL - 25	1.4
TL - 26	6.3
TL - 27	1.5
TL - 28	1.6
TL - 29	2.0
TL - 30	9.3

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Conclusion, Recommendations and Way Forward

- The Issue of regulation - The children's products needed to be strictly regulated
- BPA free products - The study also indicates that there is a consumer demand towards the BPA free products
- False Claim of manufacturers - The results exposed the false claim of the manufacturers that their products are BPA free
- Policy directive - India should take some affirmative action to regulate the use of BPA
- Research agenda - The Government of India needs to come out with a research agenda to identify the source contamination of BPA from various products and its impact on human health.

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Regulations

- In 2012 and 2013, FDA granted two petitions requesting that FDA amend its food additive regulations to no longer provide for the use of certain BPA-based materials in baby bottles, sippy cups, and infant formula packaging because these uses have been abandoned.
- World Health Organization recommend holding off on regulations that limit or ban use of BPA - November 2010

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Regulations contd..

- **Regulation of BPA in Baby feeding Bottles –**
  - **Canada is the first country to ban the import, sale and adds of BPA bottles**
  - **Banned by – USA, EU, Japan, France, Germany, Denmark, Belgium, China, Malaysia, South Africa, Turkey**
  - **Voluntary phased out by Australia**
  - **Not banned in India (Regulation in draft stage)**
- **Regulation of BPA in Baby feeding Bottles –**
  - **Prohibited By – FDA-US, Canada, European Union, Denmark, France, Argentina, Brazil, China**
  - **Voluntary Phase Out – Australia and Japan**
  - **No status - India**

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Tolerable Daily Intake (TDI)

Country/Organization	TDI (µg/kg bw/day)
European Food Safety Authority (EFSA)	50 (soon to be 5.0 µg)
FDA EPA	50
Australia	50
Chinese	50
Korean Food Safety Authority	50
India	No standards

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Thank You !

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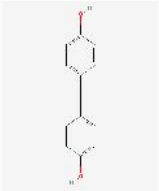
Toxicity Induced by Bisphenol A on Vital Organs in Rats

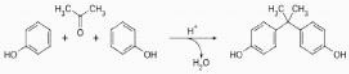
Prof. S.B.Deshpande
Department of Physiology
Institute of Medical Sciences
BHU, Varanasi

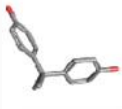
Bisphenol A

- **Organic compound with two phenol groups**
- **Prepared by the condensation of acetone with 2 phenol groups.**



2 D Structure





3 D Structure

About BPA

- **Bisphenol A – Used for manufacturing of Plastics**
- **Produced extensively- 8 billion pounds per year**
- **Synthesised initially by Dianin in 1891**
- **BPA leaches out from plastics (Type 3 and 7)**

Plastic every where

These are common scenes

Human Exposure

- Through the ingested food
- Found in breast milk due to maternal exposure
- Highest intake in infants and children.
- Inhalation in the form of dust
- By skin absorption
- In India, hot food and beverages are packed in plastics.

Arch Environ Contam Toxicol (2011) 61:68–73
DOI 10.1007/s00244-010-9634-y

Occurrence of Bisphenol A in Indoor Dust from Two Locations in the Eastern United States and Implications for Human Exposures

Sadan N. Loganathan · Kurunthachalam Kannan

Environmental Science & Technology
ARTICLE
pubs.acs.org/est

Widespread Occurrence of Bisphenol A in Paper and Paper Products: Implications for Human Exposure

Chunyang Liao and Kurunthachalam Kannan*

Environmental Science & Technology
ARTICLE
pubs.acs.org/est

High Levels of Bisphenol A in Paper Currencies from Several Countries, and Implications for Dermal Exposure

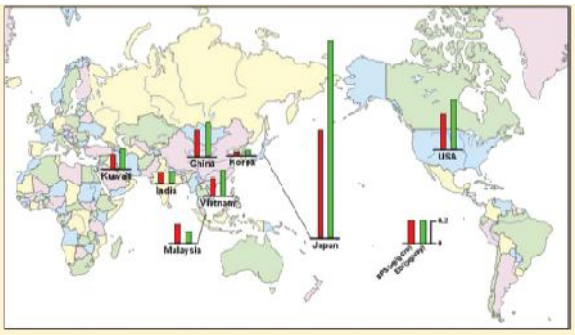
Chunyang Liao and Kurunthachalam Kannan*

Environmental Science & Technology
Article
pubs.acs.org/est

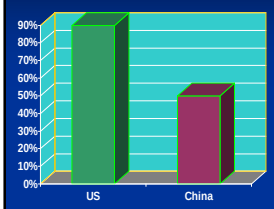
Bisphenol S in Urine from the United States and Seven Asian Countries: Occurrence and Human Exposures

Chunyang Liao,[†,‡] Fang Liu,[†] Husam Alomirah,[§] Vu Duc Loi,[∥] Mustafa Ali Mohd,[⊥] Hyo-Bang Moon,[#] Haruhiko Nakata,[∇] and Kurunthachalam Kannan[†,▼]

BPS level in urine



BPA in humans



- The high urinary levels are correlated with obesity, heart diseases and liver defects
- Infants are most exposed

Calafat et al., 2004, 2008; He et al., 2009; Melzer at el., 2010

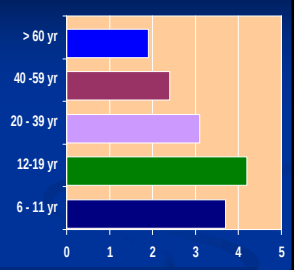
Estimated daily BPA consumption levels

AGE	BPA consumed (µg/kg bw/day)
0 – 6 months (Formula fed)	1 -11
0 – 6 months (Breast fed)	0.2 – 1
6 – 12 months	1.65 – 13
1.5 – 6 years	0.043 – 14.7
Adults	0.008 – 1.5
Occupational	0.043 – 100

National Toxicology Program, U.S. Department of Health and Human Services

Current levels of urinary BPA

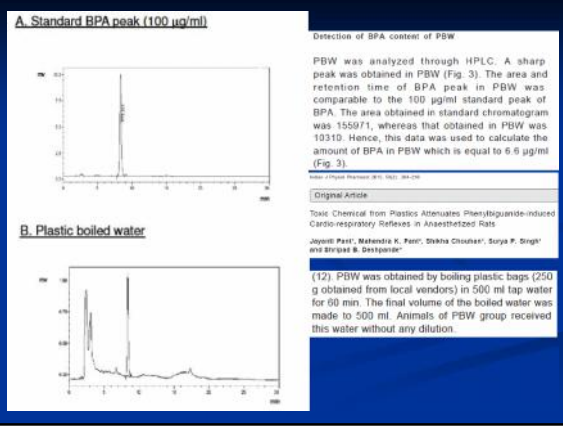
Age (years)	Urinary levels of BPA (µg/L)
6 – 11	3.7
12 – 19	4.2
20 – 39	3.1
40 – 59	2.4
> 60	1.9
Males	2.4
Females	3.2



National Toxicology Program, U.S. Department of Health and Human Services

Scenario in India

- Carrying hot food materials in plastic bags
- Hot beverages are also carried in them
- These plastic bags are recycled a number of times
- No data regarding BPA levels in Indian population



Detection of BPA content of PBW

PBW was analyzed through HPLC. A sharp peak was obtained in PBW (Fig. 3). The area and retention time of BPA peak in PBW was comparable to the 100 µg/ml standard peak of BPA. The area obtained in standard chromatogram was 155971, whereas that obtained in PBW was 10310. Hence, this data was used to calculate the amount of BPA in PBW which is equal to 6.6 µg/ml (Fig. 3).

Original Article

Toxic Chemical from Plastics Attenuates Phenylbiguanide-induced Cardio-respiratory Reflexes in Anaesthetized Rats

Jayanti Pant[1], Mahendra K. Pant[1], Shikha Chauhan[1], Surya P. Singh[1] and Shripad B. Deshpande[1]

(12). PBW was obtained by boiling plastic bags (250 g obtained from local vendors) in 500 ml tap water for 60 min. The final volume of the boiled water was made to 500 ml. Animals of PBW group received this water without any dilution.

BPA being endocrine disruptor has reported to have effects on

- **Reproduction,**
- **Mammary gland,**
- **Prostate**
- **Urinary Tract**
- **Developmental toxicity**
- **Behavioral alterations**

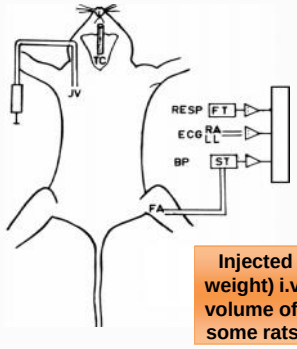
The effects of BPA on various organ systems are not available

Therefore this study was conducted to ascertain the acute and chronic effects of BPA and Plastic boiled water (PBW) on lung, heart, liver, kidney and uterus.

LD 50 of BPA in adult female rats

Route	Dose mg/kg
Intraperitoneal	841.0
Intravenous	35.3 (Lethal dose = 40)

Pant and **Deshpande** Ind J Exp Biol 2012; 50 :425-429.



JV = Jugular vein;
FA=Femoral artery canula
BP = Blood pressure;
ST = Stathum transducer;
ECG = Electrocardiogram;
TC = Tracheal cannula
RESP = Respiration;
FT = Force transducer;
RA = Right arm;
LL = Left limb;

Injected BPA (5-50 mg/kg body weight) i.v. slowly (5-7 min). Equi-volume of ethanol was injected in some rats which acted as control

The LD50 and lethal dose of BPA via iv route has a very narrow range.

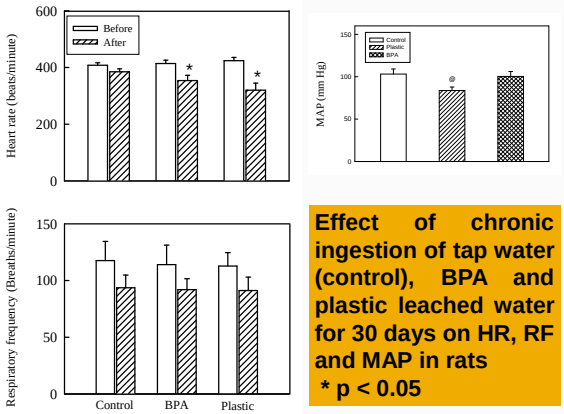
Lethal dose killed the animals within 10 min due to respiratory failure.

Pant and **Deshpande** Ind J Exp Biol 2012; 50 :425-429.

Adult female rats were given the following for 30 days with:

- **Tap water control**
- **Plastic leached water**
- **BPA containing pellets**

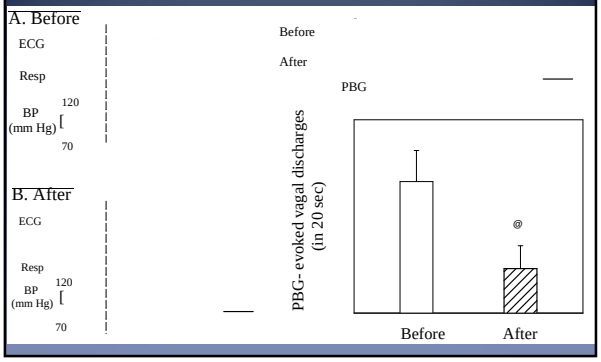
Subsequently experiments were performed



Effect of chronic ingestion of tap water (control), BPA and plastic leached water for 30 days on HR, RF and MAP in rats
*** $p < 0.05$**

- ✓ **Chronic ingestion of plastic leached water produced severe emphysematous changes in lungs.**
- ✓ **PBG-induced cardio-respiratory responses were attenuated in these rats.**
- ✓ **Hence, acute experiments were performed with BPA to understand the mechanism of attenuation of reflexes.**

BPA decreased the PBG evoked vagal afferent activity



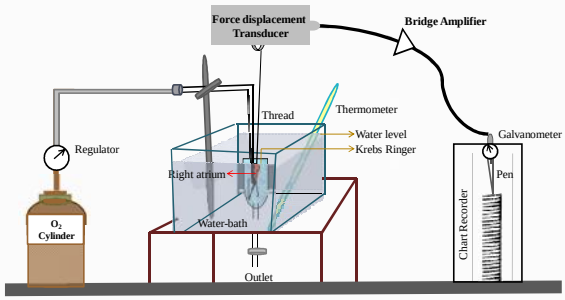
Chronic exposure to plastic leached water produced emphysematous and consolidative changes in the lungs. The pulmonary pathology may be due to the chronic attenuation of the protective respiratory reflexes by bisphenol A.

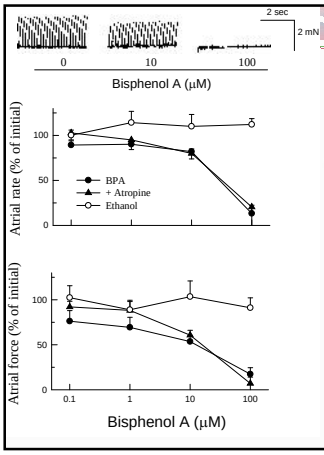
Effect of BPA on Cardiac tissue

Plastic leached water treated animals exhibited disruption of the cardiac muscle fibres and broken fibrils at intercalated disc.

Acute experiments were performed to understand the functional changes in cardia.

SET-UP USED FOR RECORDING ATRIAL CONTRACTIONS





Bisphenol A (BPA) decreased the rate and force of right atrial contractions without involving cholinergic system.

J. Appl.Toxicol. 31: 698, 2011

Results

- The results demonstrate structural changes associated with BPA or plastic water on heart.
- The observations demonstrate that BPA depressed the contractility of spontaneously beating atria *in vitro*.
- Further, our data reveal the involvement of NO-guanylyl cyclase pathway for the BPA-induced decrease in atrial contractions.

Students
Jayanti Pant
Pratibha Ranjan
Mahendra K Pant

Thank You

The deleterious effects of Bisphenol A on fertility and cognition of male mice and its alleviation by *Mucuna pruriens*

Surya Pratap Singh, Ph.D.
Professor
Department of Biochemistry
Banaras Hindu University

Introduction

- Bisphenol A (**BPA**) is a chemical used primarily in the production of polycarbonate plastics and epoxy resins.
- The approximate production volume of BPA is estimated to be **3 million tons** per year.
- Over 100 tons of BPA is estimated to be **released in** the **environment** and found in huge quantities in food, **water bodies, landfills** and other related areas.
- Exposure to BPA is associated with **adverse health effects** on humans and laboratory animals.

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•BPA is employed to make certain plastics and epoxy resins. BPA-based plastic is clear and tough, and is made into a variety of common consumer goods, such as water bottles, sports equipment, CDs, and DVDs.

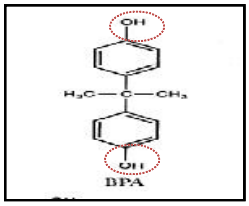
•Epoxy resins containing BPA are used to line water pipes, as coatings on the inside of many food and beverage cans and in making thermal paper such as that used in sales receipts.

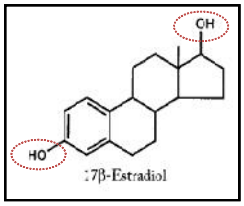
•In 2011, an estimated 10 billion pounds of BPA chemical were produced for manufacturing polycarbonate plastic, making it one of the highest volume of chemicals produced worldwide.

•BPA exhibits hormone-like properties that raise concern about its suitability in some consumer products and food containers.

•Since 2008, several governments have investigated its safety, which prompted some retailers to withdraw polycarbonate products.

•The FDA has ended its authorization of the use of BPA in baby bottles and infant formula packaging, based on market abandonment, not safety. The European Union and Canada have banned BPA use in baby bottles.





- The core structure of BPA resembles that of the natural estrogen, consisting of two phenolic rings joined by a bridging carbon.
- Thus, BPA [2,2-(4,4-dihydroxydiphenol) propane] acts as a xenoestrogen and hence it has been placed in the group of compounds called endocrine disruptor (ED). EDs adversely effect the normal functioning of the endocrine system.

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What are the risks associated?

- BPA molecules are bound by "ester bonds" to form polycarbonate plastic.
- The bond is disrupted by heat and acidic or basic conditions that release BPA into food or beverages in contact with the plastics.

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What are the possible health effects of bisphenol A (BPA) on humans?

- **Reproductive disorders** - scientists from Brigham and Women's Hospital showed that BPA exposure can affect egg maturation in humans.
- **Male impotence** - Dr De-Kun Li, a reproductive and perinatal epidemiologist at Kaiser Permanente's Division of Research in Oakland, California, reported in the journal Human Reproduction that BPA exposure may raise the risk of erectile dysfunction. Sexual desire and problems with ejaculation were also linked to BPA exposure among men.
- **Asthma** - A US study suggested a link between increasing asthma rates and a particular threshold of BPA.

- **Chemotherapy** - University of Cincinnati scientists found that BPA exposure may reduce chemotherapy treatment efficacy.
- **Breast cancer** - A Yale School of Medicine study found a possible increase in breast cancer risk among females exposed to BPA and DES (Diethylstilbestrol) in the womb.
- **Brain function, memory, learning** - US researchers linked BPA exposure to loss of connections between brain cells in primates, potential problems with memory and learning, as well as depression.
- **Women's eggs** - Californian researchers found that exposure to bisphenol A may affect the quality of a woman's eggs retrieved for in vitro fertilization (IVF).

- **Heart disease (females)** - BPA can cause heart disease in women, scientists at the University of Cincinnati found.
- **Heart disease in adults** - another US study linked BPA exposure to diabetes and heart disease in adults.
- **Sex hormones in men** - an August 2010 study linked BPA exposure to changes in sex hormones in men.
- **Type 2 diabetes** - A UK study linked higher levels of urinary BPA to type 2 diabetes, cardiovascular disease and liver-enzyme abnormalities.

Daily Intake of Bisphenol A

Population	Estimated daily bisphenol A intake, µg/kg body wt/day. Table adapted from the National Toxicology Program Expert Panel Report.[11]
Infant (0–6 months) formula-fed	1–11
Infant (0–6 months) breast-fed	0.2–1
Infant (6–12 months)	1.65–13
Child (1.5–6 years)	0.043–14.7
Adult (general population)	0.008–1.5
Adult (occupational)	0.043–100

Mucuna pruriens

- *Mucuna pruriens* (Mp) is called **'Kewach'** in Hindi and **'Kappikachhu'** in Sanskrit, a herb belonging to family *Fabaceae*, is found throughout the Indian subcontinent in wet, damp and marshy areas. Its beneficial roles have been attributed to the presence of **L-DOPA**.
- **Mp** has been shown to **increase testosterone levels**, leading to deposition of protein in the muscles and increased muscle mass and strength.
- **Mp** has been used as an **effective aphrodisiac** and also known to possess antispasmodic, anti-inflammatory, antipyretic and antivenin properties.

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Objective

To elucidate the protective effect of *Mucuna pruriens* on BPA afflicted male infertility and cognitive dysfunctions.

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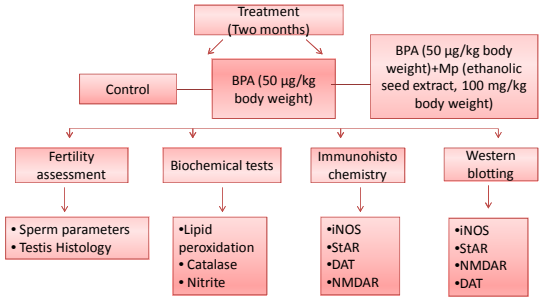
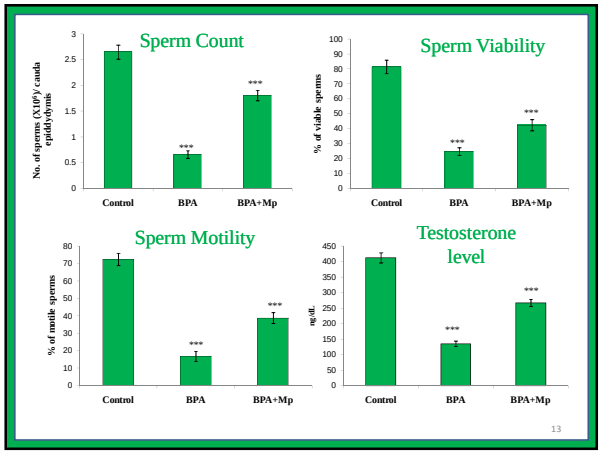
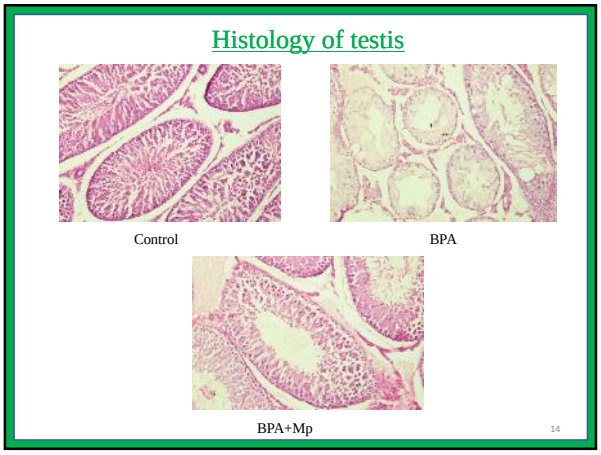
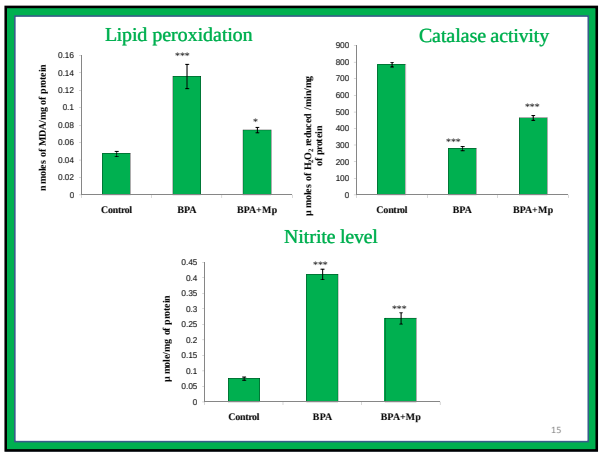
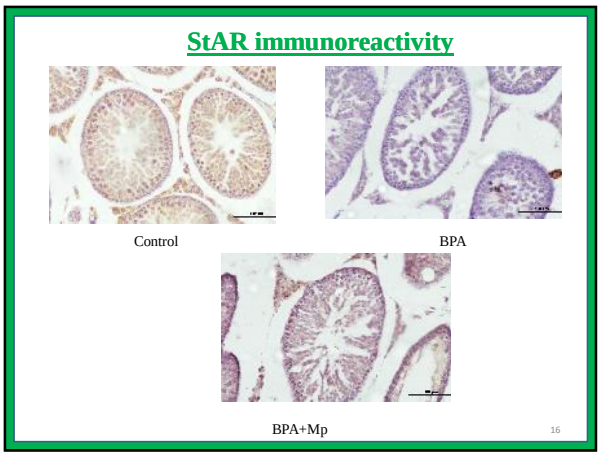
Plan of Work

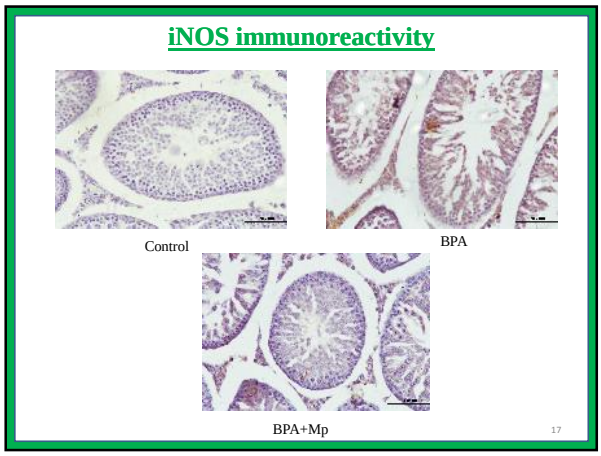


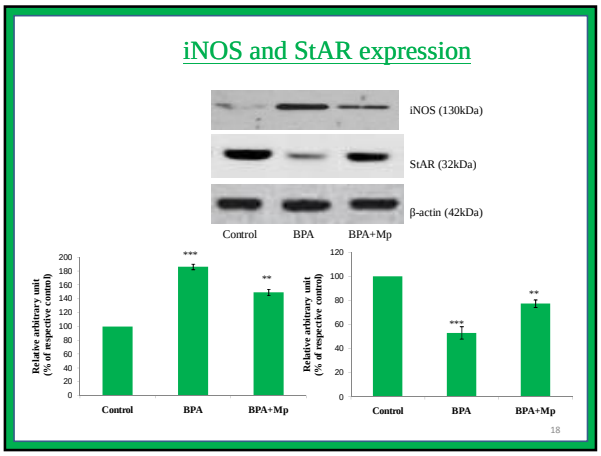

Sperm Count
No. of sperms (X10⁶)/ cauda epididydymis
Control
BPA
BPA+Mp
Sperm Viability
% of viable sperms
Sperm Motility
% of motile sperms
Testosterone level
ng/dL
13

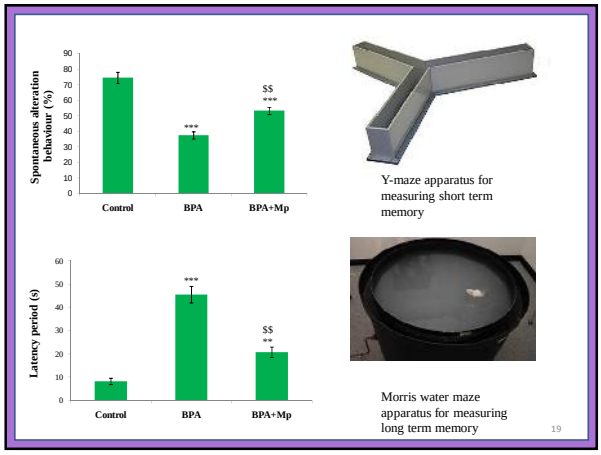

Histology of testis
Control
BPA
BPA+Mp
14

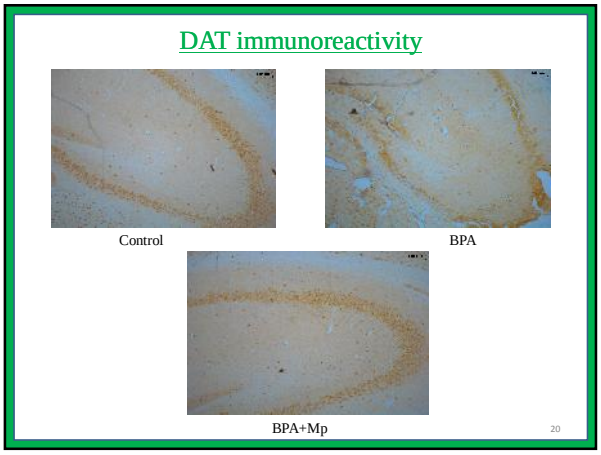

Lipid peroxidation
n moles of MDA/mg of protein
Control
BPA
BPA+Mp
Catalase activity
µ moles of H2O2 reduced /min/mg of protein
Nitrite level
µ mole/mg of protein
15

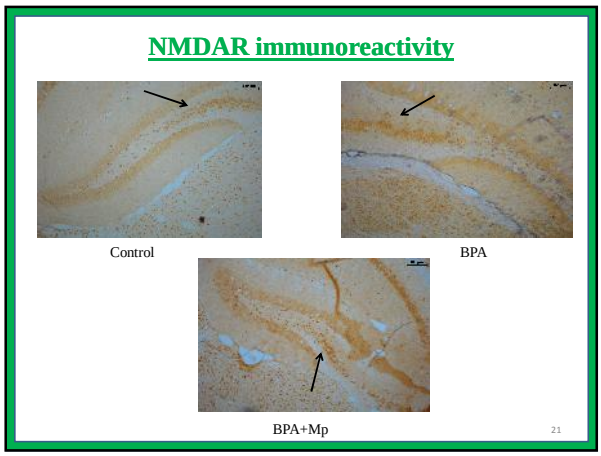

StAR immunoreactivity
Control
BPA
BPA+Mp
16

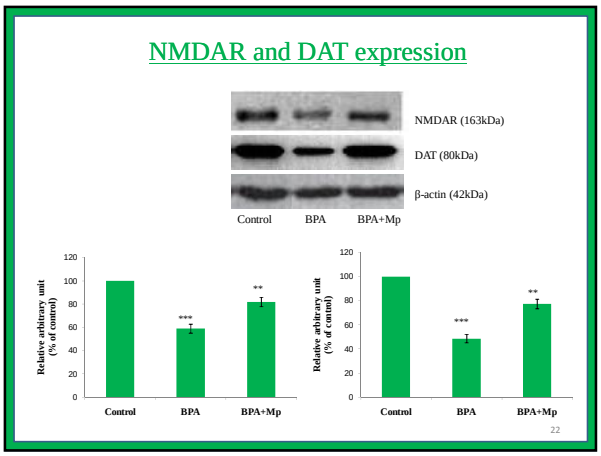

iNOS immunoreactivity
Control
BPA
BPA+Mp
17

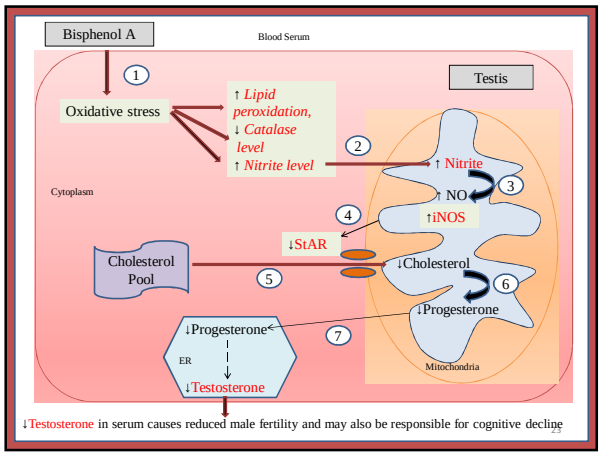

iNOS and StAR expression
iNOS (130kDa)
StAR (32kDa)
β-actin (42kDa)
Control
BPA
BPA+Mp
Relative arbitrary unit (% of respective control)
18

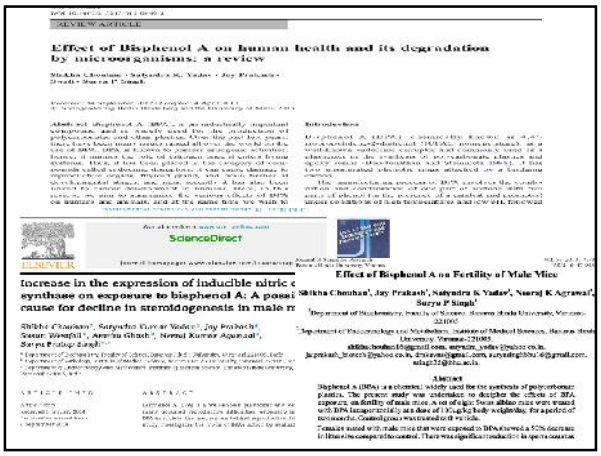

Spontaneous alteration behaviour (%)
Control
BPA
BPA+Mp
Y-maze apparatus for measuring short term memory
Latency period (s)
Morris water maze apparatus for measuring long term memory
19


DAT immunoreactivity
Control
BPA
BPA+Mp
20


NMDAR immunoreactivity
Control
BPA
BPA+Mp
21


NMDAR and DAT expression
NMDAR (163kDa)
DAT (80kDa)
β-actin (42kDa)
Control
BPA
BPA+Mp
Relative arbitrary unit (% of control)
22


Bisphenol A
Blood Serum
Testis
Oxidative stress
↑ Lipid peroxidation,
↓ Catalase level
↑ Nitrite level
↑ Nitrite
↑ NO
↑iNOS
↓StAR
Cytoplasm
Cholesterol Pool
↓Cholesterol
↓Progesterone
↓Progesterone
ER
↓Testosterone
Mitochondria
↓Testosterone in serum causes reduced male fertility and may also be responsible for cognitive decline


Effect of Bisphenol A on human health and its degradation by microorganisms: a review
Increase in the expression of inducible nitric oxide synthase on exposure to bisphenol A: A possible cause for decline in steroidogenesis in male rats
Effect of Bisphenol A on Fertility of Male Mice

How to avoid A Bisphenol exposure

- According to various consumer groups, those wishing to reduce BPA exposure should not eat/drink canned foods and drinks unless the labelling says it is BPA free.
- Scientist from the Silent Spring Institute, USA and Breast Cancer Fund, USA report that after just three days on a fresh food diet, BPA and DEHP levels in children and adults fell considerably.
- You should not microwave foods in plastic containers. Plastic containers should not be washed in the dishwasher. You should avoid cleaning plastic containers with harsh detergents.

What are the alternatives?

- BPA free and epoxy free lining of cans.
- BPA free plastic bottles and containers.
- Use of polyester resin and oleoresin for the lining of cans.
- A substitute of BPA such as BPS was earlier thought to be harmless but recent studies published in 2015 says that "based on the current literature, BPS and BPF are as hormonally active as BPA, and have endocrine disrupting effects."
- Phenol-based substitutes : Among potential substitutes for BPA, phenol-based chemicals closely related to BPA have been identified. The non-extensive list includes bisphenol E (BPE), bisphenols B (BPB), and 4-cumylphenol (HPP).

Conclusion

- The results from the present study strongly suggest that BPA has deleterious effects on reproduction of adult male mice. Significant decline in the level of testosterone reveals the **steriodogenic disruption** caused by BPA.
- The mode of action of BPA can be contributed to the increased expression of iNOS which further leads to a reduced expression of StAR thereby effecting the steriodogenesis process.
- L-DOPA content of the **plant helps** to **increase** the **testosterone level** and helps to **improve** the **spermatogenic and steroidogenic activity.**
- Further the study reveals an increase in sperm count and motility. Also Mp helps to **improve** the **DAT and NMDAR** expression in the **hippocampal region** of mice brain which helps in improving the performance of the mice at spatial navigation tasks.

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Acknowledgements

- Satyndra Kumar Yadav
- Jay Prakash
- Shikha Chouhan
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- Ashok Yadav
-
  - CSIR New Delhi
  - DST New Delhi
  - UGC-UPE

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Recent Publications

•Pant J, Pant MK, Chouhan S, **Singh SP**, Deshpande SB. (2015). Toxic Chemical from Plastics Attenuates Phenylbiguanide-induced Cardio-respiratory Reflexes in Anaesthetized Rats. Indian J **Physiol Pharmacol**. 2015 Apr-Jun; 59 (2): 204-10.

•The Gut Microflora and its Metabolites Regulate the Molecular Crosstalk between Diabetes and Neurodegeneration. Susan Westfall1, Nikita Lomis1, **Surya Pratap Singh2**, Si Yuan Dai1 and Satya Prakash1* (2015**) J Diabetes Metab**. 6(8): 1-16. doi:10.4172/2155- 6156.1000577

•Chauhan, A. K., Ahmad, A., **Singh, S. P**., Kumar, A. (2015). Nucleotide sequencing of *dszABC* genes from a desulfurizing strain *Mycobacterium phlei* SM120-1. *International Journal of* ***Applied Biochemistry*** **3**:1-5.

•Chouhan S, Yadav SK, Prakash J, Westfall S, Ghosh A, Agarwal NK, **Singh SP** (2015) Increase in the expression of inducible nitric oxide synthase on exposure to bisphenol A: A possible cuase for decline in steroidogenesis in male mice. **Environ Toxicol Pharmacol.** 13 (1): 404-416.

•Yadav, S.K., Prakash, J., Chouhan, S., Westfall, S., Verma, M., Singh, T., Singh, S.P. (2014) Comparison of the Neuroprotective Potential of *Mucuna pruriens* Seed Extract with Estrogen in MPTP-Induced PD mice model. Neurochem Int.65:1-13..

•Yadav, S.K., Prakash, J., Chouhan, S., Singh, S.P. (2013) Mucuna pruriens seed extract reduces oxidative stress in nigrostraital tissue and improves neurobehavioural activity in paraquat-induced Parkinsonian mouse model. Neurochem. Int. 62:1039-1047.

About Toxics Link

Toxics Link is an Indian environmental research and advocacy organization set up in 1996, engaged in disseminating information to help strengthen the campaign against toxics pollution, provide cleaner alternatives and bring together groups and people affected by this problem.

Toxics Link's Mission Statement - "*Working together for environmental justice and freedom from toxics. We have taken upon ourselves to collect and share both information about the sources and the dangers of poisons in our environment and bodies, and information about clean and sustainable alternatives for India and the rest of the world*"

Toxics Link has a unique expertise in areas of hazardous, medical and municipal wastes, international waste trade, and the emerging issues of pesticides, Persistent Organic Pollutants (POPs), hazardous heavy metal contamination etc. from the environment and public health point of view. We have successfully implemented various best practices and have brought in policy changes in the aforementioned areas apart from creating awareness among several stakeholder groups.

Toxics Link
for a toxics-free world

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RESEARCH TEAM:
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Dr. Prashant Rajankar
Kritika Mathur

AGENDA

Conference on Endocrine Disrupting Chemicals

Junipar, India Habitat Centre, New Delhi

4th February 2016

09.30 am – 10.00 am	**REGISTRATION**
10.00 am – 10.45 am	**INAUGURATION SESSION** Mr. Ravi Aggarwal – Director, Toxics Link Prof. Alok Dhawan – Director, Indian Institute of Toxicological Research, Lucknow Ms. Lesley Onyon – Regional Advisor, WHO South-East Regional Office Mr. Satish Sinha – Associate Director, Toxics Link
10.45 am – 11.00 am	**TEA**
1. Understanding Endocrine Disrupting Chemicals (EDCs) **Chair:** Dr. Alok Dhawan**,** Director, Indian Institute of Toxicological Research, Lucknow **Co Chair:** Dr. K.S Sachdeva, Addl DDG, Ministry of Health and Family Welfare, GOI	
11.00 am – 11.20 am	EDCs and Environmental and Health impacts **Dr. David Gunnarson**, Swedish Society for Nature Conservation
11.20 am – 11.40 am	Effects of early life exposure to Bisphenol A on reproduction and understanding its mechanism of action **Dr. Geeta Vanage,** National Inst. for Research in Reproductive Health, ICMR
11.40 am – 12.00 pm	Global initiatives to regulate EDCs (Yet to confirm) **Alexandra Caterbow,** International POPs Elimination Network
2. Research Studies on EDCs in India -I **Chair: Prof. Y. K. Gupta**, AIIMS, New Delhi **Co Chair:** Mr Sanyal, Consumer Voice	
12.00 pm – 12.20 pm	Toxics Link research study on BPA in Baby bottles, **Dr. Prashant Rajankar,** Toxics Link
12.20 pm – 12.40 pm	Research on BPA by **Prof. S. B Deshpande**, IMS, Varanasi
12.40 pm – 01.00 pm	**DISCUSSION**
01.00 pm – 02.00 pm	**LUNCH**

AGENDA

3. **Research Studies on EDCs in India -II** **Chair:** Mr Sunil Sharma, Director, Ministry of Chemicals **Co Chair:** Mr Bharat Singh, MoEFCC	
02.00 pm – 02.20 pm	Erosion of Cellular Transcription Memory by Endocrine Disrupting Chemicals **Prof. R.K. Tyagi**, Special Centre for Molecular Medicine, JNU
02.20 pm – 02.40 pm	Pesticides as EDCs and its impacts on human health **Dr. R.S. Dhaliwal**, ICMR, New Delhi
02.40 pm – 03.00 pm	Research on Impacts of pharmaceutical EDCs **Dr. Ashok P Manekar**, Institute of Science, Mumbai
03.00 pm – 03.20 pm	Endocrine disruptors: Their impacts on health at exposures **Dr Partho Roy**, Indian Institute of Technology, Roorkee
03.20 pm – 03.45 pm	**DISCUSSION**
03.45 pm – 04.00 pm	**TEA**
04:00 pm – 05.00 pm	**PANEL DISCUSSION** Mr. Satish Sinha, Toxics Link Prof. Y. K. Gupta, AIIMS, New Delhi Dr. L.M. Srivastav, Sir Gangaram Hospital Bureau of Indian Standards Industry

RSVP:

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