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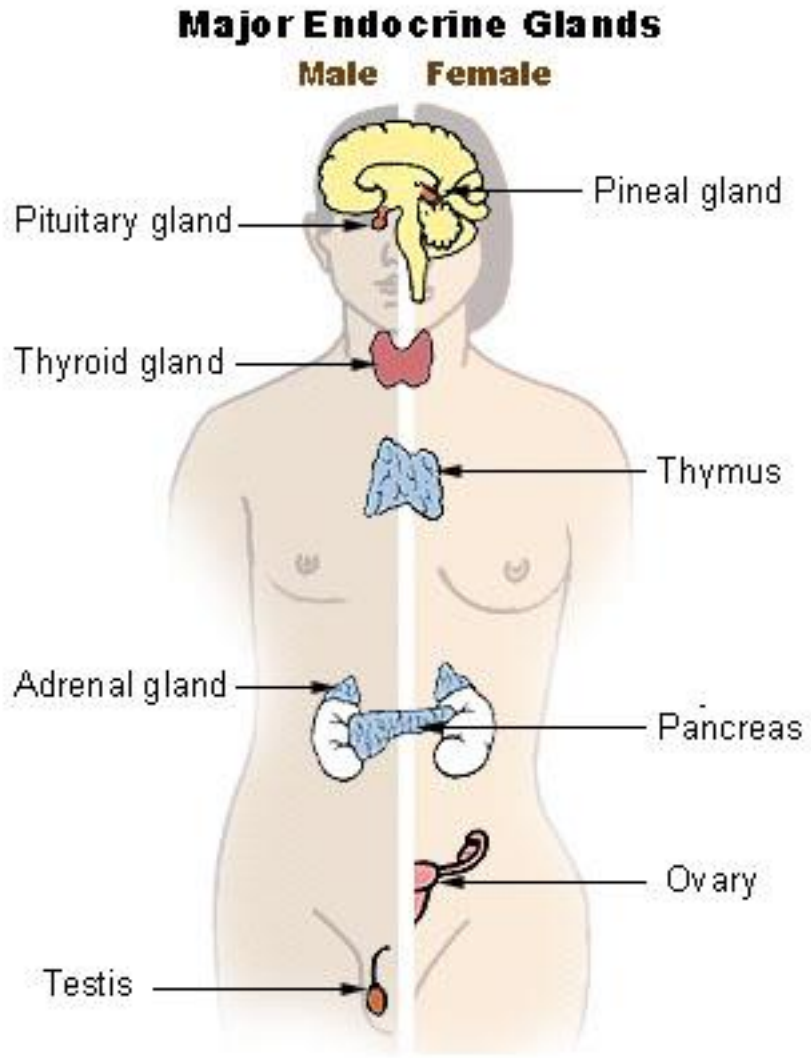
"*In vitro* bioassays for the identification and toxicological study of endocrine disrupting food contaminants"

The Endocrine System

- Hormonal signaling system
- Steroidogenesis
- Nuclear receptors
- Critical cellular processes



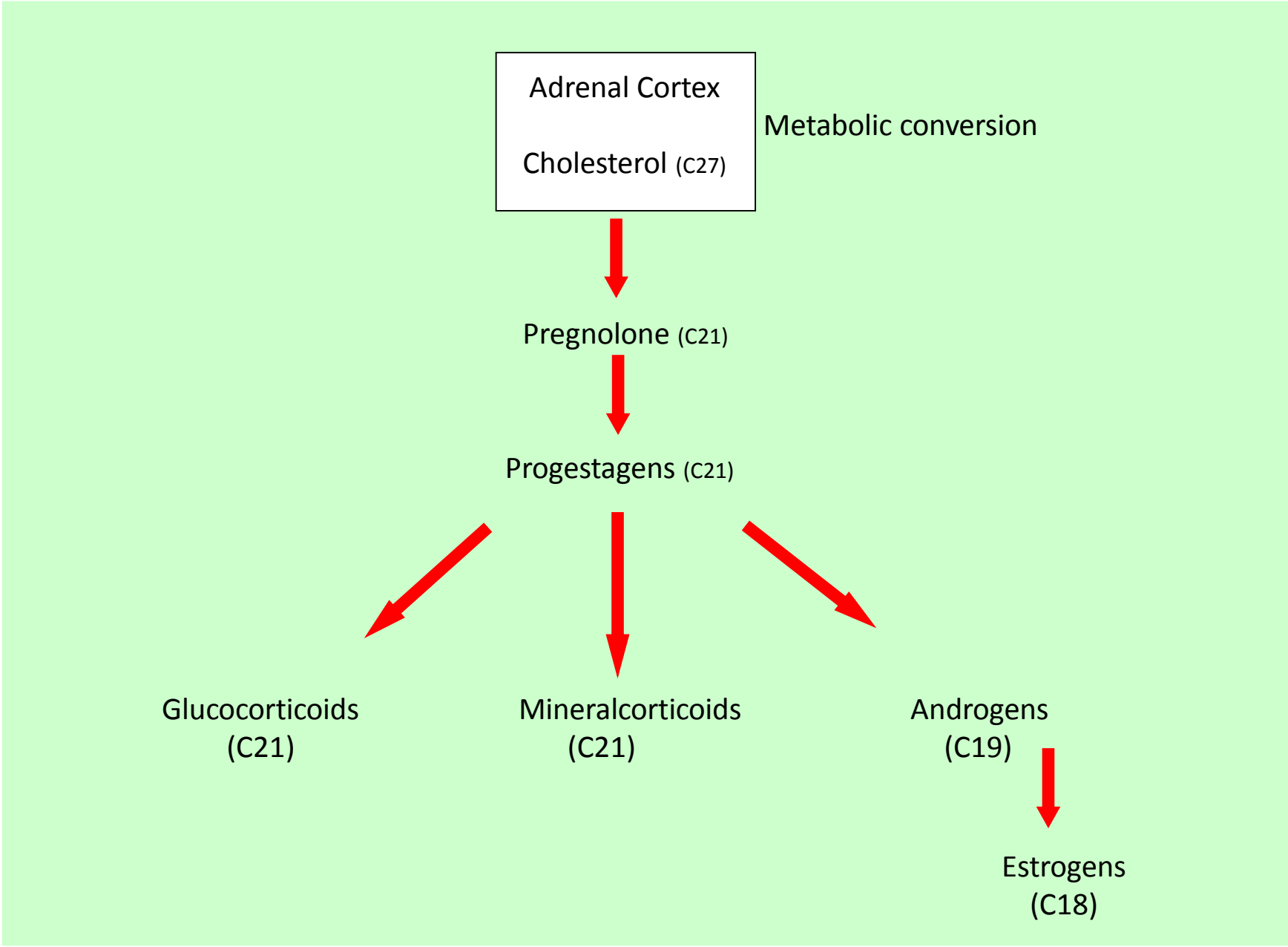
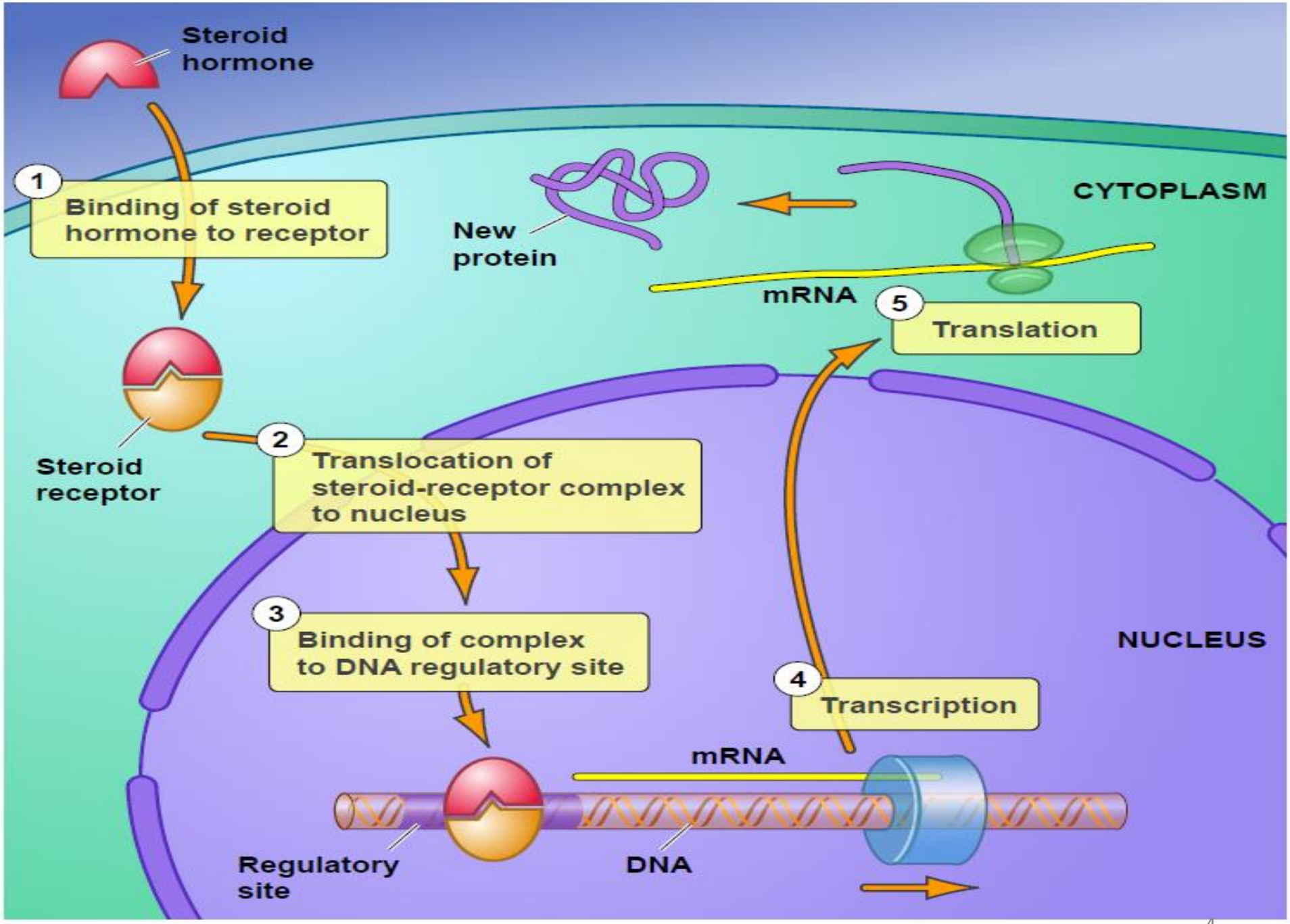


Figure. Steroid Hormone Metabolism (Steroidogenesis)



Source: "Module 6." <http://www.thepepproject.net>

Endocrine Disruptor

IPCS/WHO 2002 definition:

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

Trends in Analytical Chemistry, Vol. 30, No. 2, 2011

In vitro bioassays for the stud… of endocrine-disrupting foo… additives and contamina…

L. Connolly, E. Ropstad, S. Verhaegen

Endocrine-disrupting chemicals (EDCs) are capable of interfering wi… , acting … and through a wide variety of mechanisms. Unwanted exposure … m of adve… especially when exposure is during critical windows of develop… d to be amon… of inadvertent exposure to EDCs, so there is an important … s in these matrices.

We describe *in vitro* bioassays that can complement … order to detect un… describe their action, emphasizing assays that can m… signaling or hormon… outline both validated and unvalidated *in vitro* ass… ific community for dete… the effects of EDCs, and discuss their possible … e conclude by identifying gaps in the current battery of *in vitro* assays available for EDCs … development and validation.

Keywords: Endocrine disruptor; Food additive; Food … o bioassay; Leydig; Mycotoxin; Nuclear receptor; Phytoestrogen; Reporter-gene assay

Biological activity

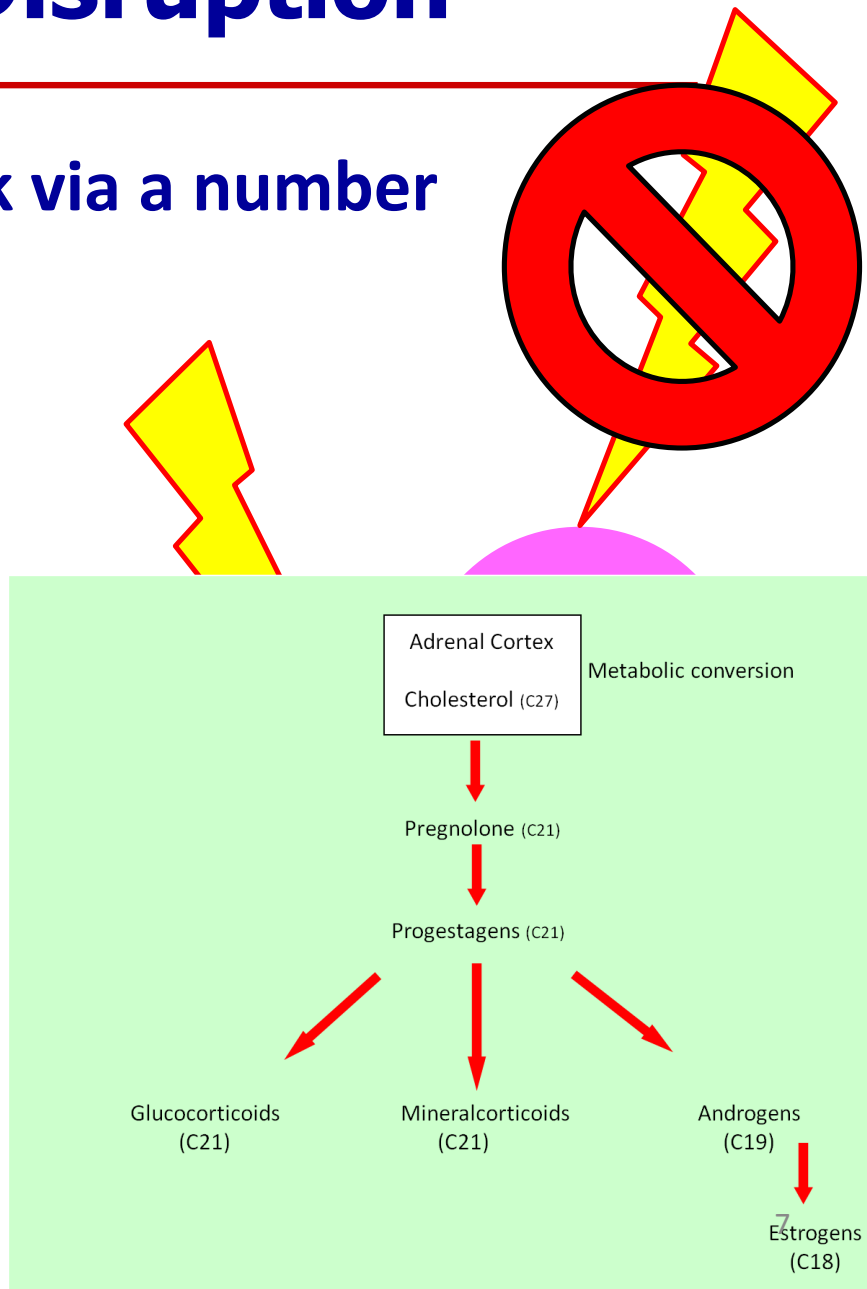
- …cted …lysis
- …ffect directed analysis
- Mechanistic study
- Mixture analysis
- Risk assessment

Endocrine Disruption

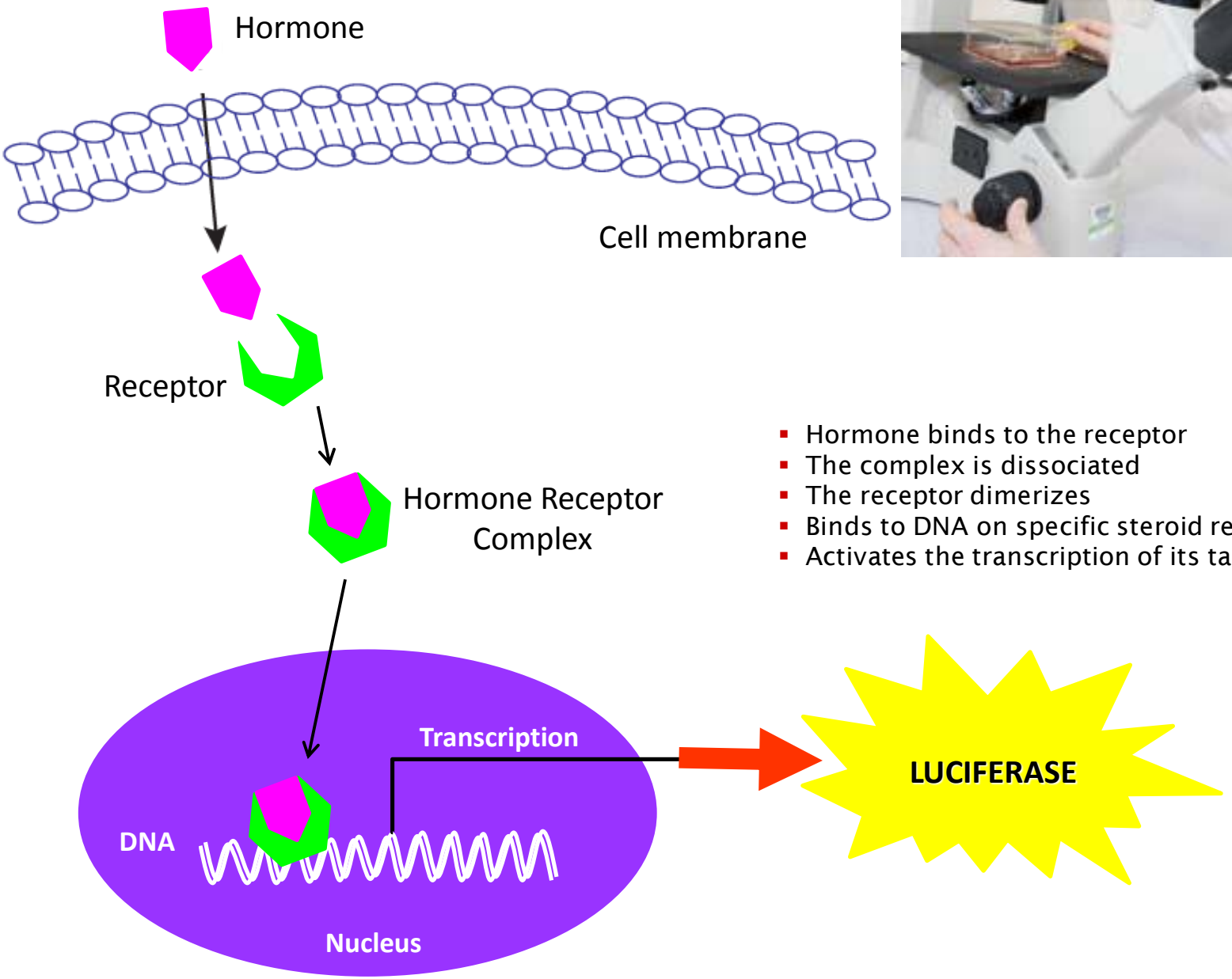
Endocrine disruptors can work via a number of mechanisms:

1. **Mimic natural hormones and:**
   a) **Activate** cellular receptors - agonists
   b) **Block** cellular receptors - antagonists

2. **Interfere with hormone production pathways by disrupting hormone:**
   a) Production
   b) Secretion
   c) Control system



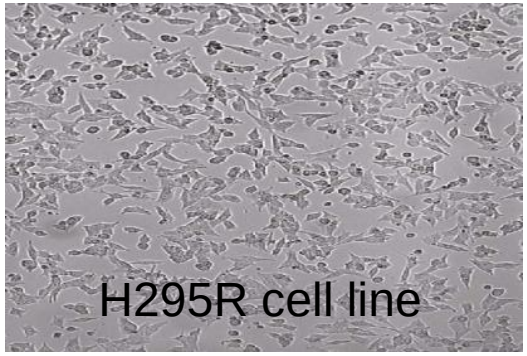
Reporter Gene Assay

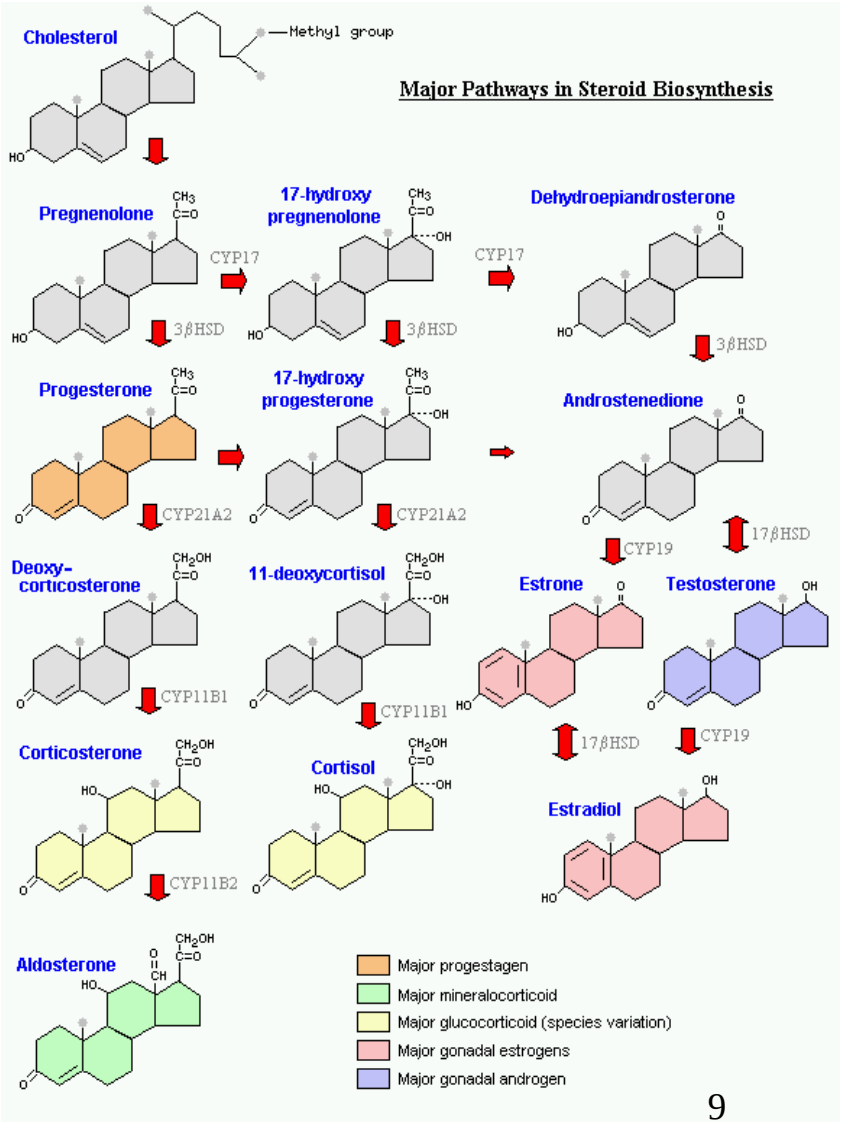


- Hormone binds to the receptor
- The complex is dissociated
- The receptor dimerizes
- Binds to DNA on specific steroid response elements
- Activates the transcription of its target genes

H295R Steroidogenesis Assay

- **Established from human female adrenocortical carcinoma**
- **Maintains ability to secrete all major adrenocortical steroid hormones**
  - progestagens
  - Androgens and estrogens
  - glucocorticoids and mineralocorticoids
- **Expresses most of the important steroidogenic enzymes**
  - CYP11A, CYP11B, CYP17, CYP19, CYP21

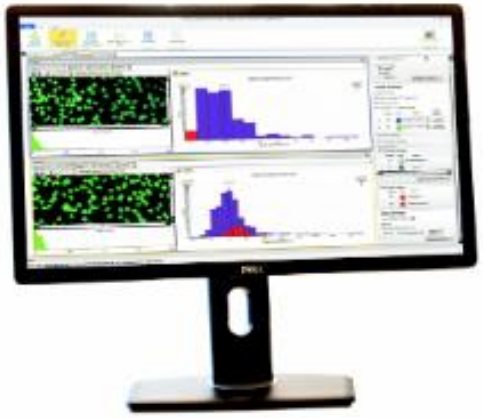




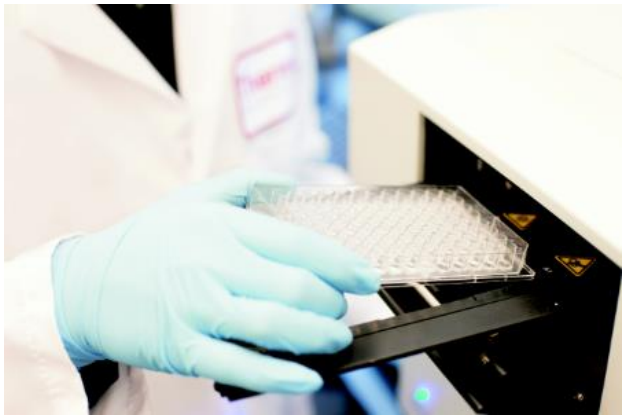
High Content Analysis (HCA)

The emerging technology called High content analysis (HCA) is a highly powerful **multi-parameter** bio-analytical based tool incorporating fluorescent microscopy with automated *in vitro* cell analysis software.

HCA provides assays that have high sensitivity and specificity for **pre-lethal toxicity** and can be used as a high throughput-screening tool to monitor the toxicity, **endocrine disruption** and **biological effects** of compounds on exposed cells.



CellInsight



Multiple toxicological end-points

Sub-lethal toxicity

Mechanistic pathway studies

Interlinked toxicity pathways

GR-Redistribution HCA Assay

The recombinant U2OS cells express the human glucocorticoid receptor (GR) fused to the C-terminus of enhanced green fluorescent protein (EGFP).

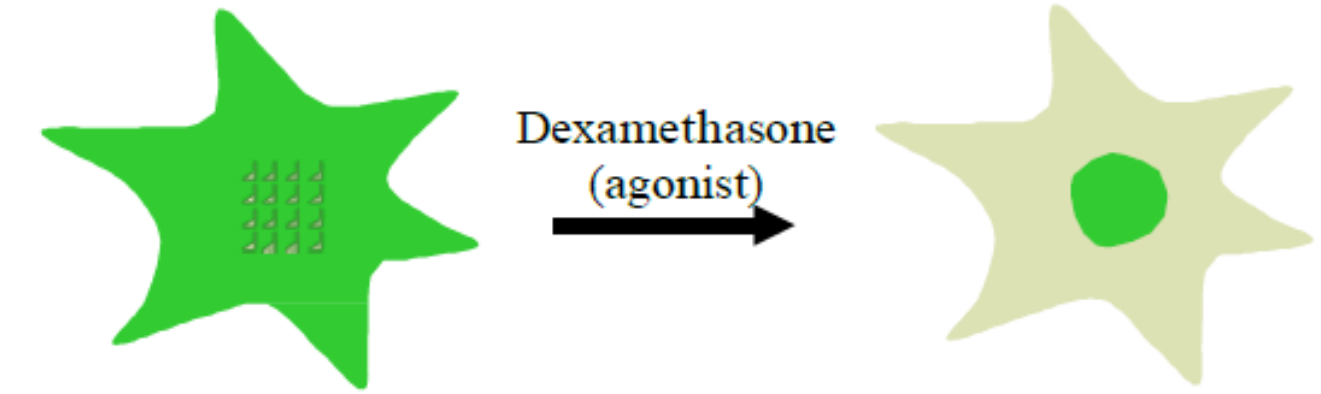


Figure 1: Illustration of the GR translocation.

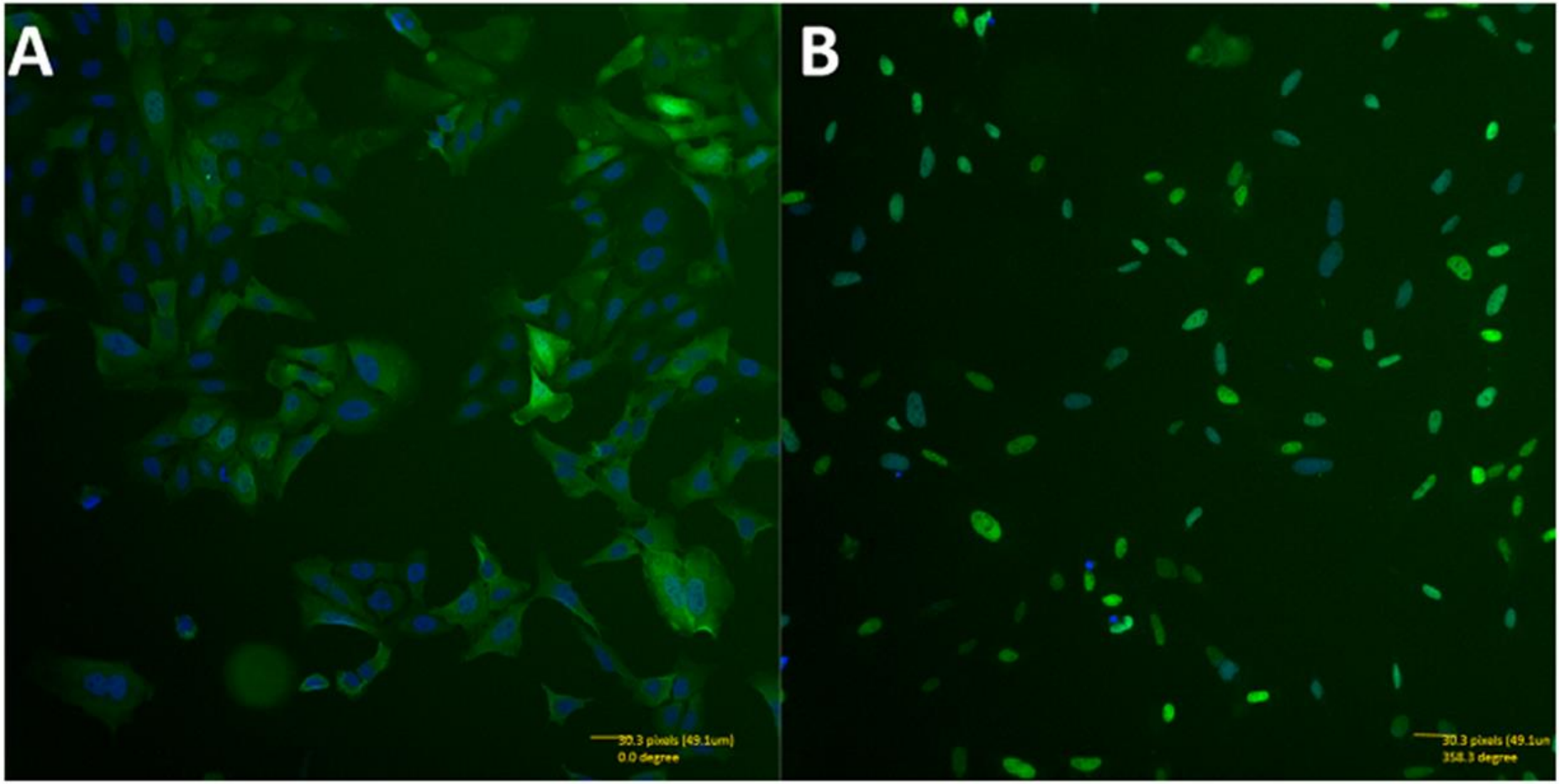
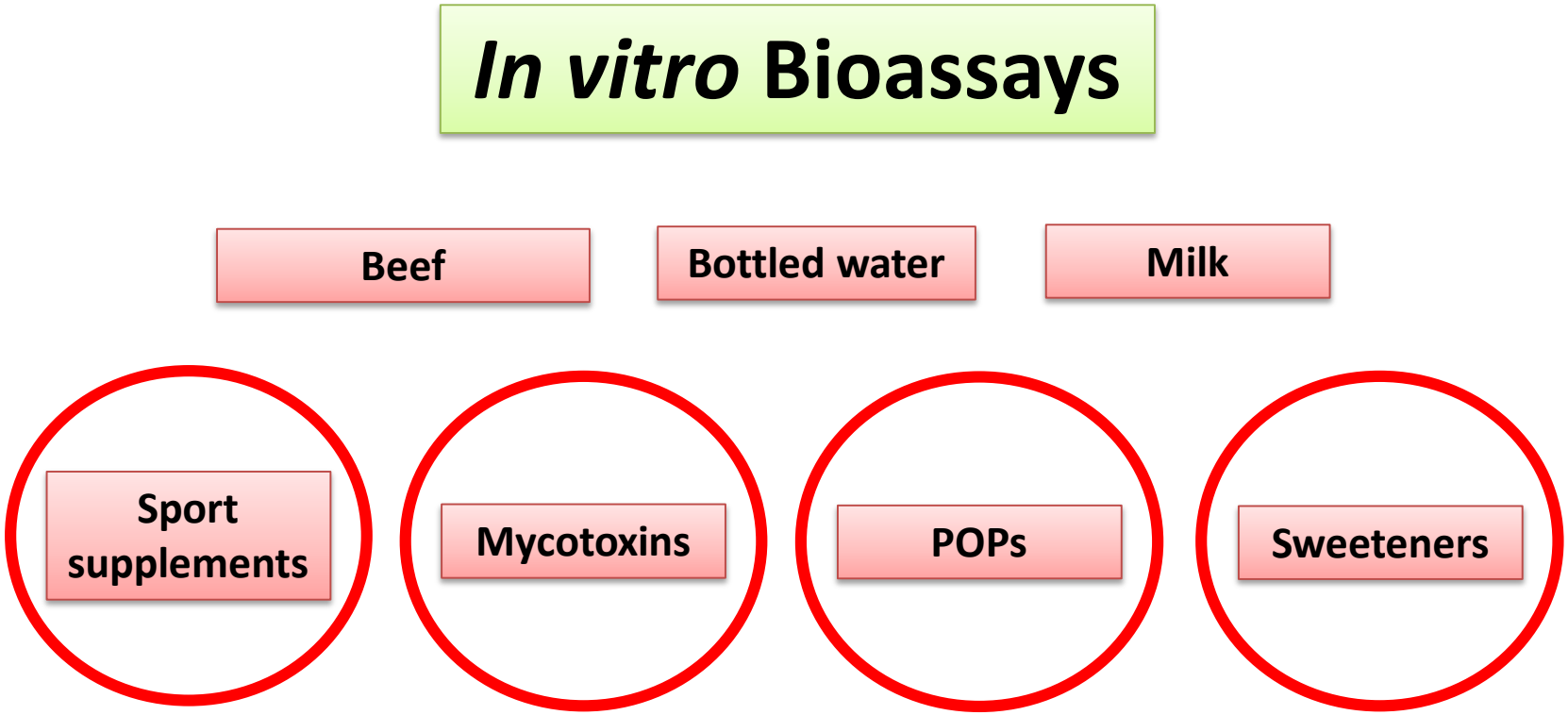


Figure 4: Example of GR RA images: (A) negative control - DMSO (B) positive control – 11.4 ng/ml cortisol. In the inactive state, the GR (images show this receptor tagged with enhanced GFP) is found in the cytoplasm in complex with heat shock proteins. Upon ligand binding, the GR translocates to the nucleus, dimerizes, and acts as a transcription factor to regulate the expression of its target genes. (Blue stain is Hoechst nuclear stain).

Food Contaminants/Additives



Emerging Endocrine Disruptors
Real life exposure mixtures

Food Chemistry 159 (2014) 157–165

Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/foodchem

Estrogenic endocrine disruptors present in sports supplements. A risk assessment for human health

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Reporter gene assay
Risk assessment

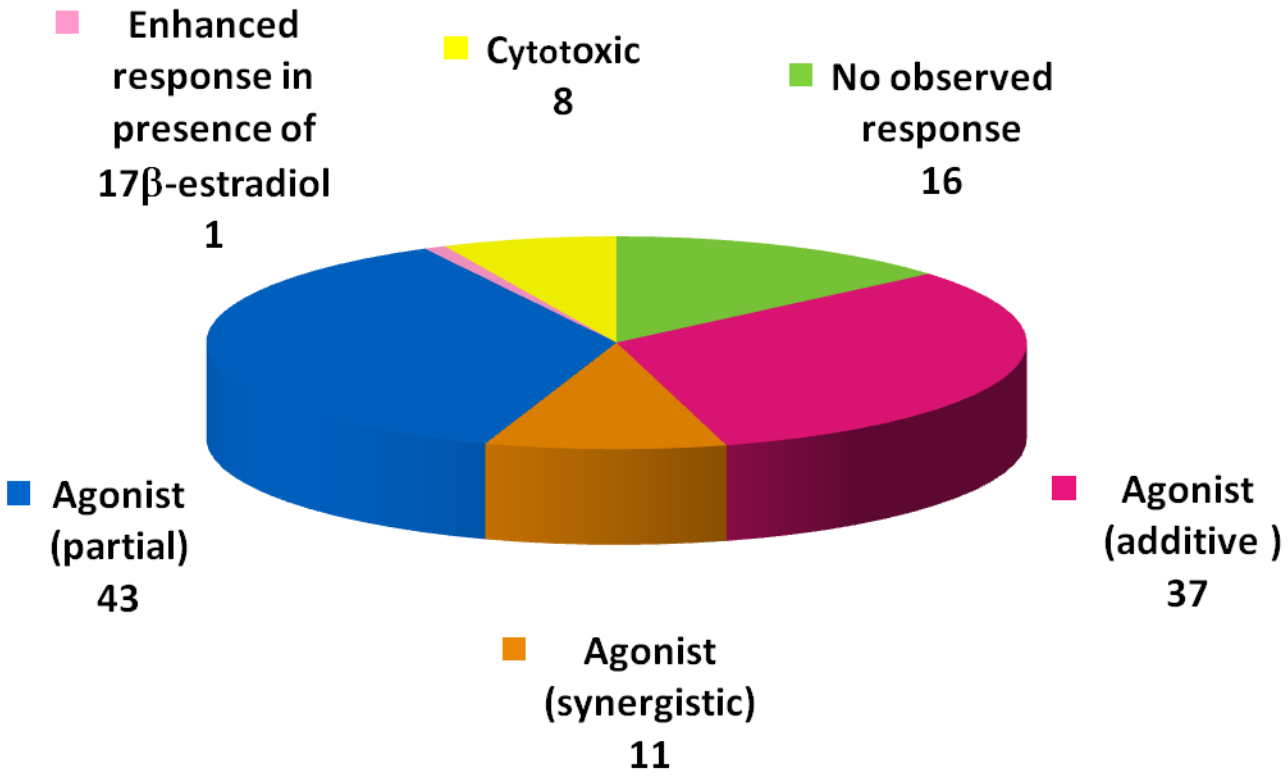
ABSTRACT

Sports supplements are becoming a regular dietary addition for consumers who view such products as a means of improving their health and performance. Previously estrogenic endocrine disruptors (EDs) were detected in 80% of 116 sports supplements investigated by biological *in vitro* reporter gene assays (RGAs). The aim of this study was to quantify the hormonal activity in 50 of these sports supplement samples using a validated estrogen RGA and perform an exposure and risk assessment for human health.

Results showed that 17β-estradiol equivalent levels were higher than those reported as being present in the typical human omnivore diet in 33 of the sports supplements and higher than the acceptable daily intake (ADI) in 13 of these products. The highest activity samples presented a potential to influence the human daily exposure to 17β-estradiol like activity in various risk groups with a predicted hormonal impact of greatest concern in young boys and postmenopausal women.

In conclusion, consumers of sports supplements may be exposed to high levels of estrogenic EDs.

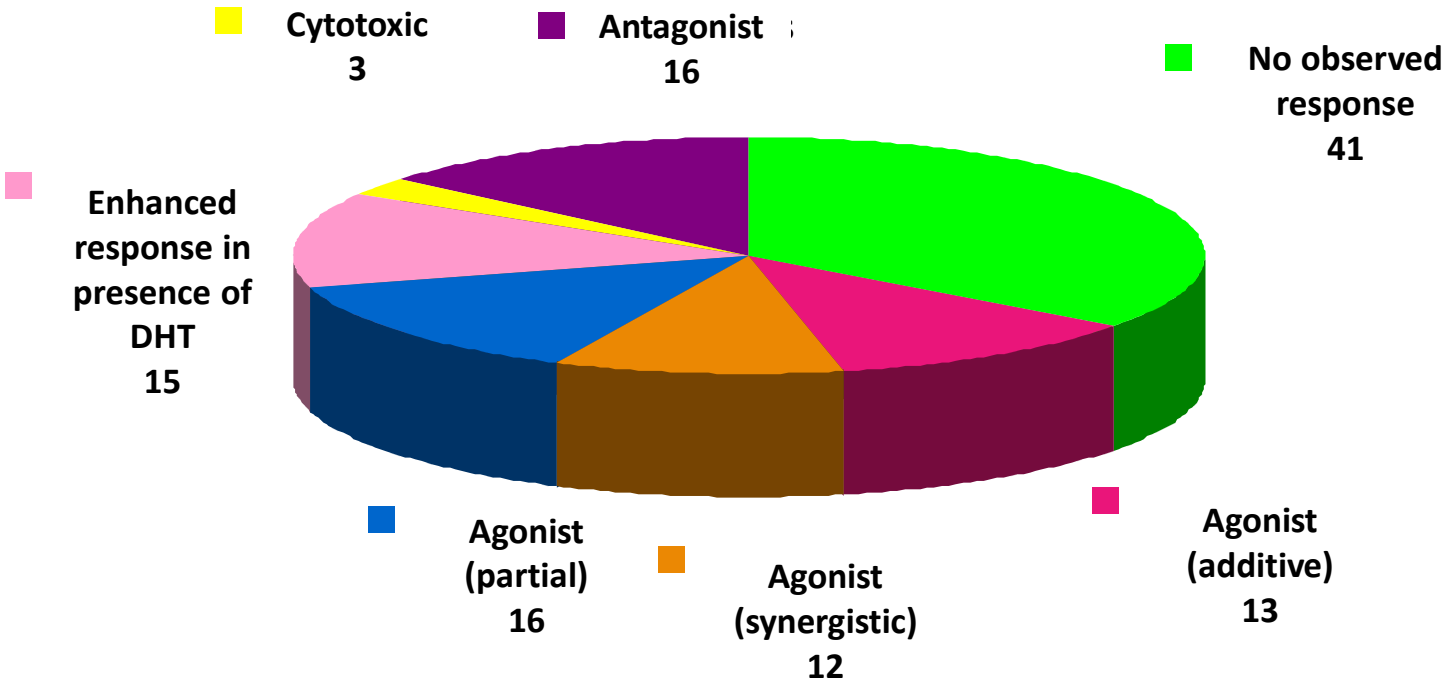
Estrogen Results



Detection capability: 17β-estradiol 0.0026 ng mL^{-1}

86% of samples showed some form of response

Androgen Results



Detection capability: DHT 0.025 ng mL^{-1}

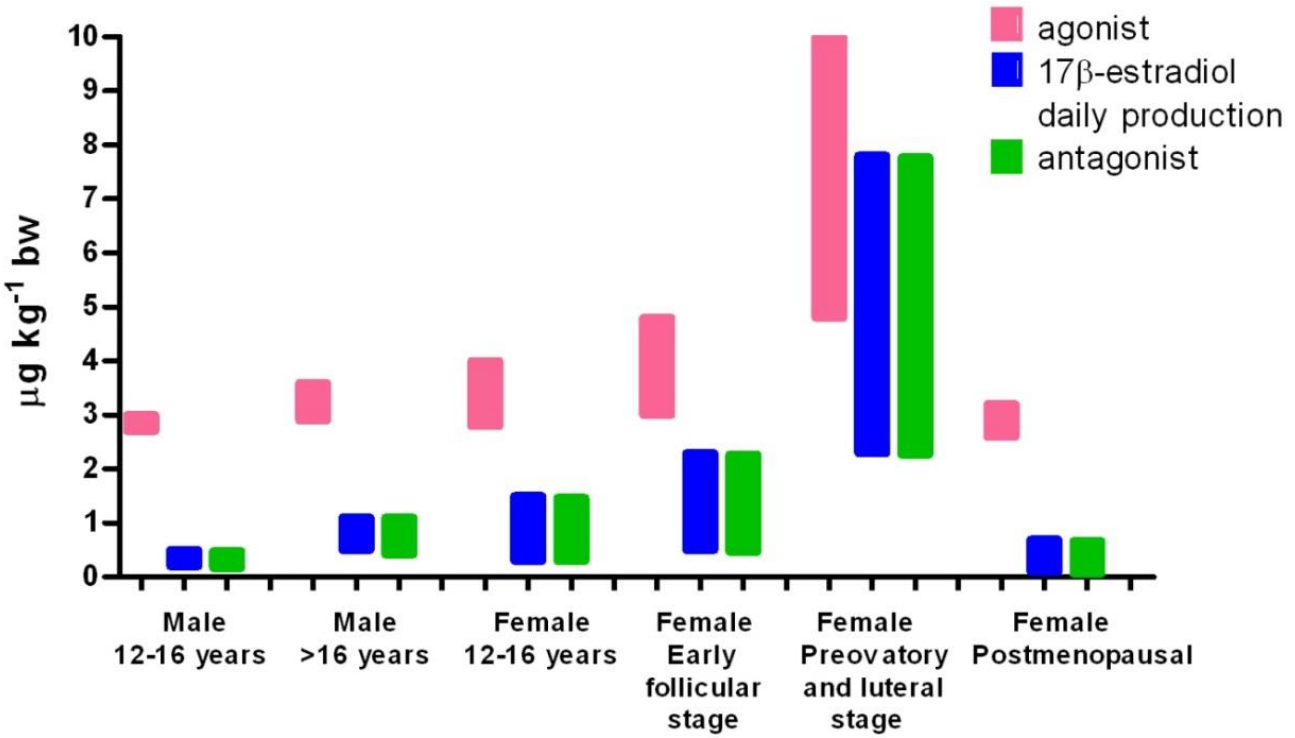
65% of samples showed some form of response

Exposure Assessment

The levels of endocrine disruptors detected were compared with the acceptable daily intakes (ADIs) of 17β-estradiol and testosterone established by JECFA:

- 13 samples exceeded the ADI limitation set for 17β-estradiol
- 4 samples exceeded the ADI limitation for testosterone

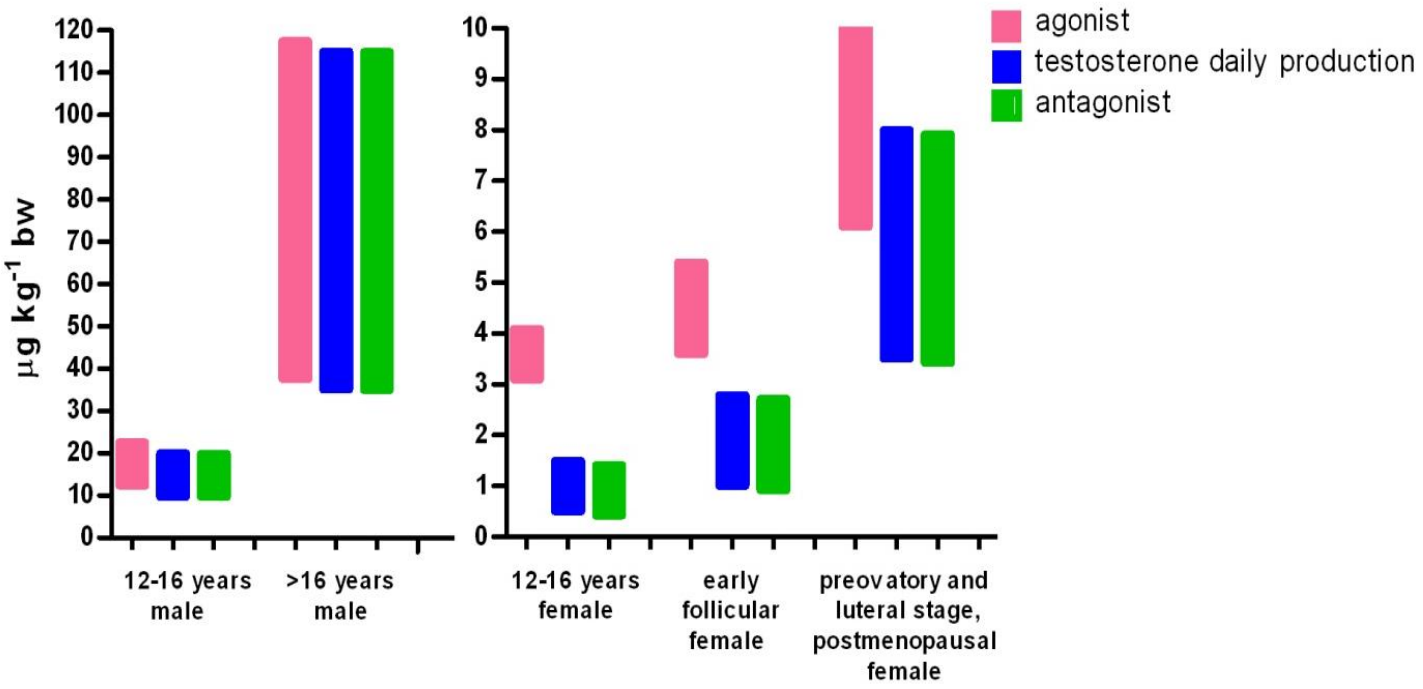
Estrogen Risk Assessment



! Young boys and post-menopausal women !

(17β-estradiol levels may be increased by up to 20 fold)

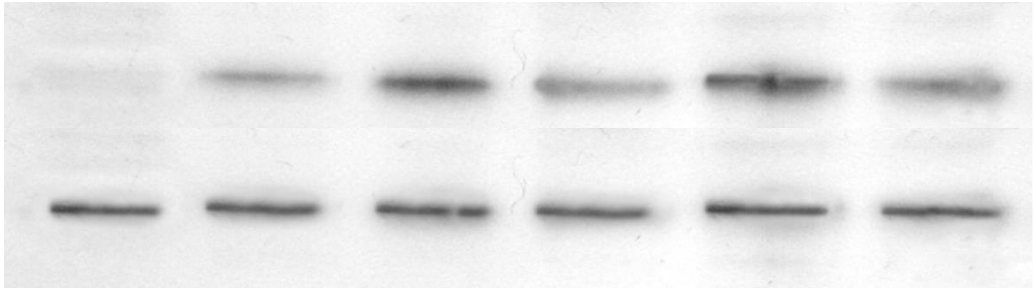
Androgen Risk Assessment



! Girls and women in the early follicular stage of the menstrual cycle !

(When testosterone levels are at their lowest)

Androgen Risk Assessment



1	2	3	4	5	6
MMV-Luc untreated	TARM-Luc untreated	TARM-Luc + DHT	TARM-Luc + DHT + antagonist	TARM-Luc + Sample extract	TARM-Luc + Sample extract + antagonist

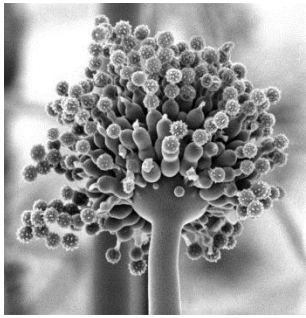
110kDa
(Androgen receptor)

37kDa
(GADPH control)

Conclusions

- Sport supplement samples (116) were screened in the estrogen and androgen assay.
- A range of endocrine disruptor activity types were detected.
- The identity of the active compounds remain to be confirmed.
- Western blot analysis identified the presence of endocrine disruptors capable of enhancing the production of the androgen receptor.
- Exposure assessment:
    - 13 samples exceeded the ADI limitation set for 17β-estradiol.
    - 4 samples exceeded the ADI limitation for testosterone.
- Risk assessment:
    - Estrogen - young boys and post menopausal women.
    - Androgen - girls and women in the early follicular stage of the menstrual cycle.

Mycotoxins in food and feed



- **Aflatoxins**: B1, B2, G1, G2, M1
- **Fusarium Moulds:**
  - **Trichothecenes** T-2 and HT-2 toxin, diacetoxyscirpenol (DAS), deoxynivalenol or vomitoxin (DON), nivalenol
  - **Resorcyclic acid lactones** zearalanone
  - **Fumonisins** B1, B2, B3
- **Ochratoxin A**
- **Patulin**

European Commission Legislation

- Tolerable Daily Intakes (TDI) established
- Strict legislative limits for residual levels

Toxicology Letters 206 (2011) 210–217

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Endocrine disrupting effects of zearalenone, alpha- and beta-zearalenol at the level of nuclear receptor binding and steroidogenesis

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[c] Sokoine University of Agriculture, Morogoro, Tanzania
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H295R
Sterodogenesis
Endocrine disruptor

ABSTRACT

The mycotoxin zearalenone (ZEN) is a secondary metabolite of fungi which is produced by certain species of the genus *Fusarium* and can occur in cereals and other plant products. Reporter gene assays incorporating natural steroid receptors and the H295R steroidogenesis assay have been implemented to assess the endocrine disrupting activity of ZEN and its metabolites α-zearalenol (α-ZOL) and β-zearalenol (β-ZOL).

α-ZOL exhibited the strongest estrogenic potency (EC_{50} 0.022 ± 0.001 nM), slightly less potent than 17-β estradiol (EC_{50} 0.015 ± 0.002 nM). ZEN was ~70 times less potent than α-ZOL and twice as potent as β-ZOL. Binding of progesterone to the progestagen receptor was shown to be synergistically increased in the presence of ZEN, α-ZOL or β-ZOL.

ZEN, α-ZOL or β-ZOL increased production of progesterone, estradiol, testosterone and cortisol hormones in the H295R steroidogenesis assay, with peak productions at 10 μM. At 100 μM, cell viability decreased and levels of hormones were significantly reduced except for progesterone. β-ZOL increased estradiol concentrations more than α-ZOL or ZEN, with a maximum effect at 10 μM, with β-ZOL (562 ± 59 pg/ml) > α-ZOL (494 ± 60 pg/ml) > ZEN (375 ± 43 pg/ml). The results indicate that ZEN and its metabolites can act as potential endocrine disruptors at the level of nuclear receptor signalling and by altering hormone production.

Receptor activity

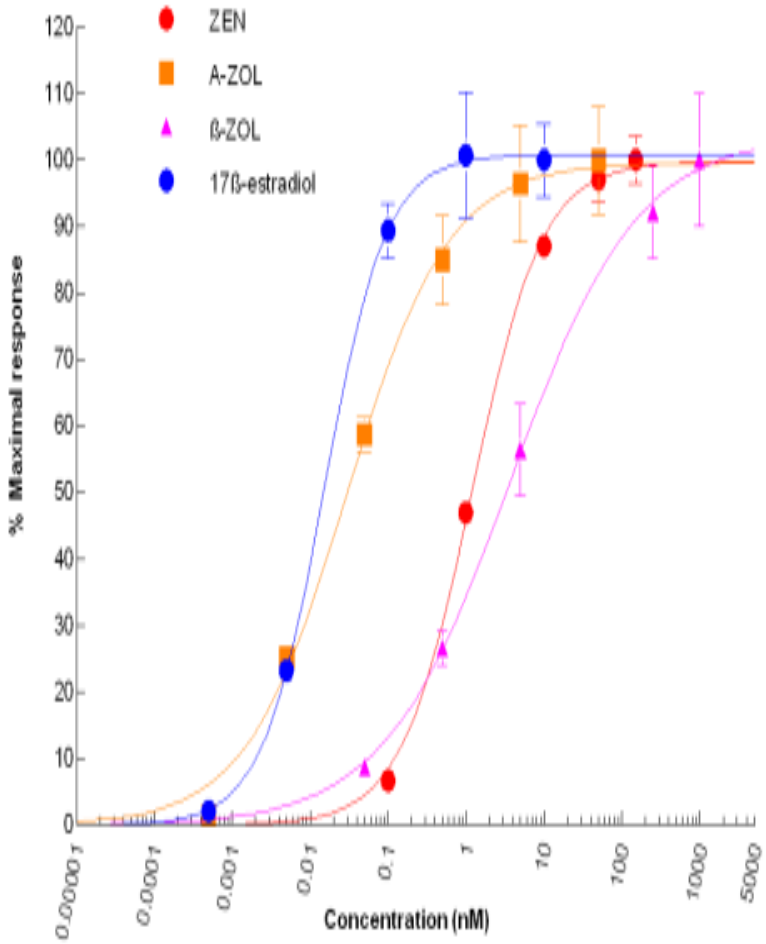

Figure 3. Estrogenic response of 17β-estradiol, ZEN, α-ZOL and β -ZOL using the MMV-Luc (estrogen responsive) RGA cell line (*n* = 3).

Progesterone Receptor

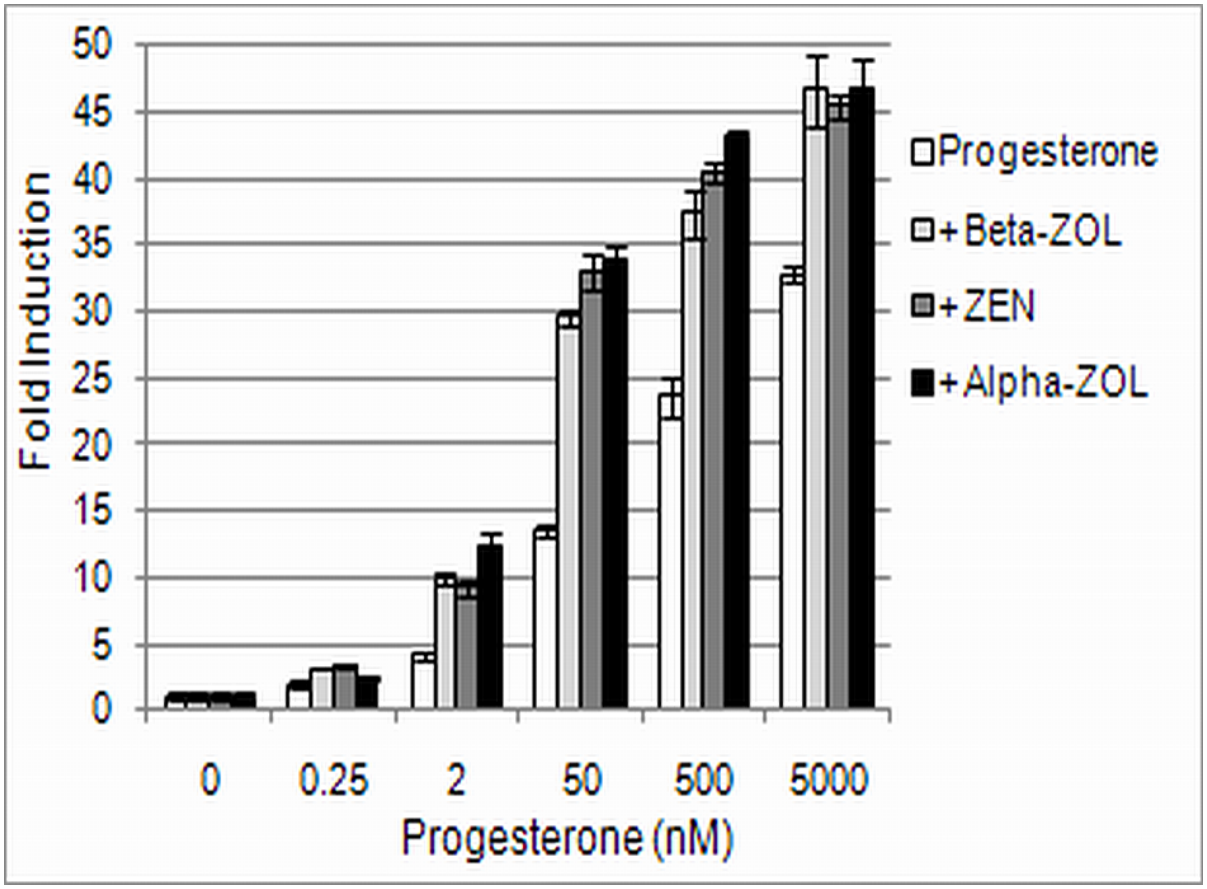

Figure 5. The effect 25 nM of ZEN, α-ZOL or β-ZOL on target gene activation by progesterone (0.25-5000 nM) through its relevant receptor in the TM-Luc (progesterone responsive) cell line. The response is measured as fold induction when compared with the negative control (*n*=3).

Progesterone Receptor

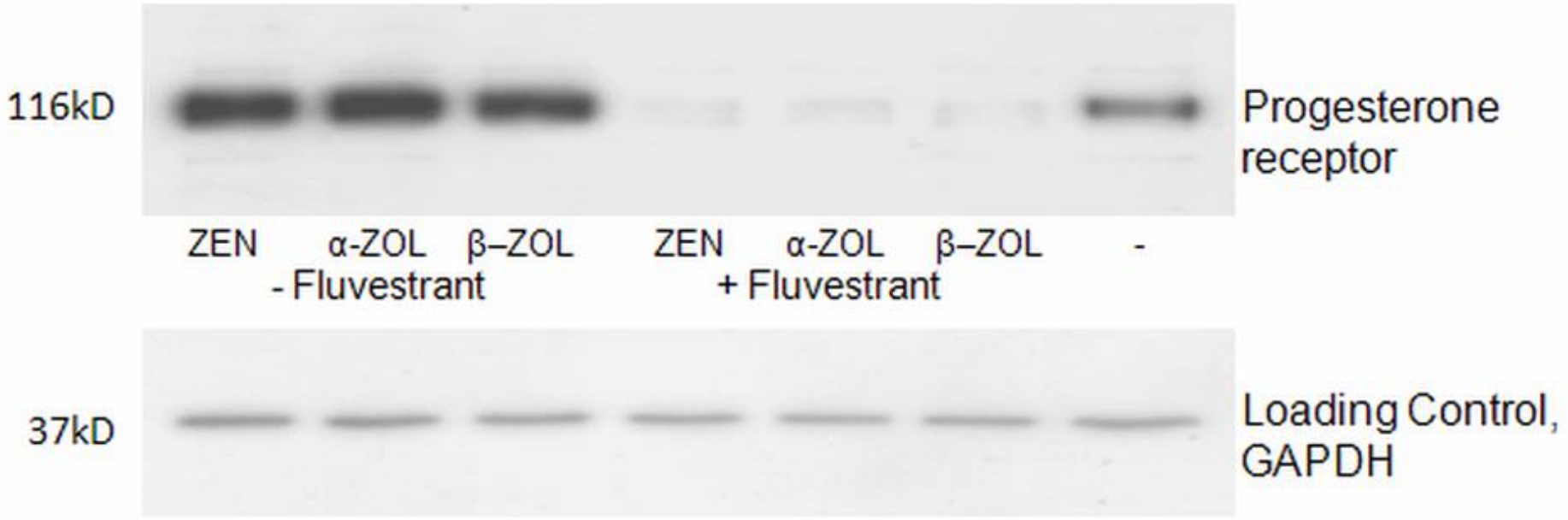

Figure 7. Western Blot analysis of the progesterone receptor protein in the TM-Luc cell line after treatment with 25 nM ZEN, α-ZOL or β-ZOL with and without 1 µM of the estrogen receptor antagonist fluvestrant. GAPDH protein is shown as a control for protein loading.

Steroidogenesis

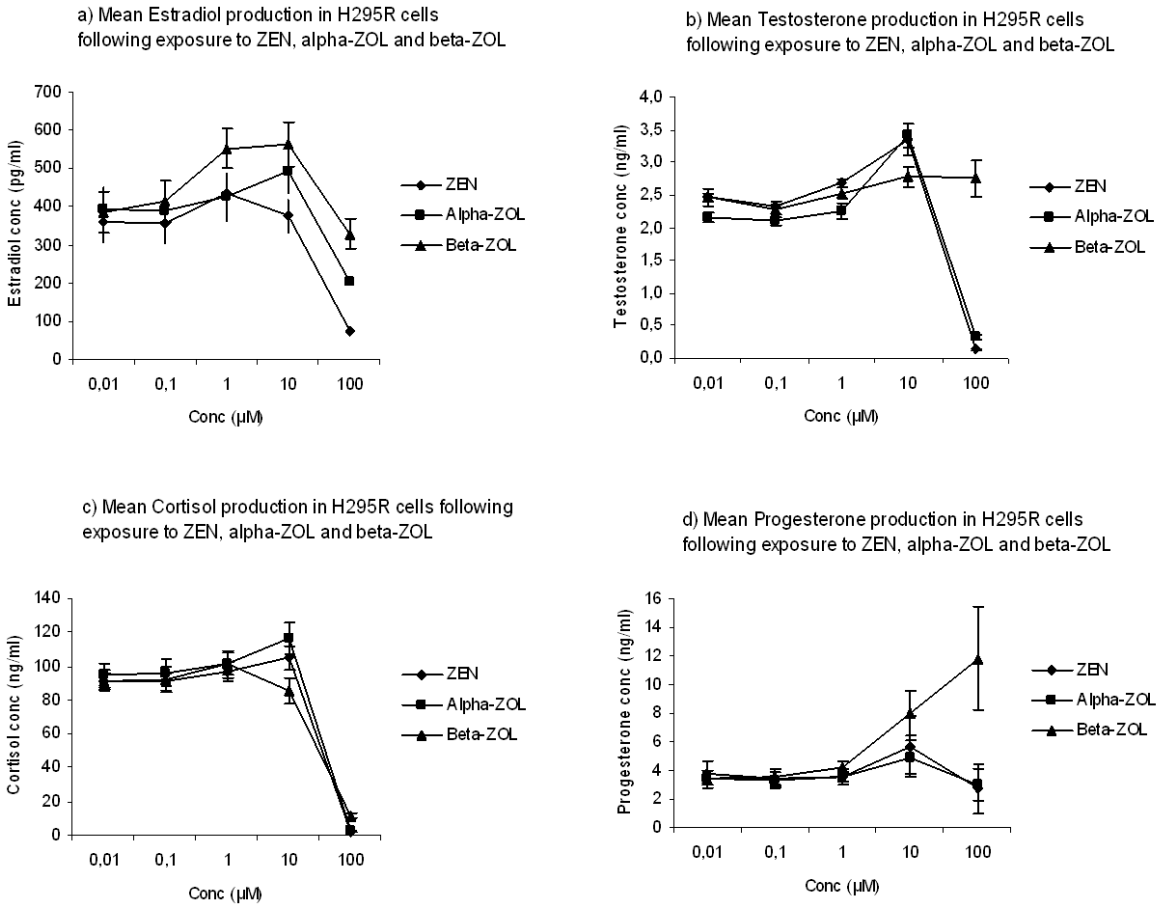

Figure 6. Mean a) estradiol, b) testosterone, c) cortisol and d) progesterone hormone production by H295R cells following exposure to 0.01 µM, 0.1 µM, 1.0 µM, 10 µM and 100 µM of ZEN, α-ZOL and βZOL.

Conclusions

- ZEN and its metabolites can affect the endocrine system at different levels.
- **Receptor level:** α-ZOL, ZEN and β-ZOL showed an estrogenic agonistic effect in order of increasing activity.
- This estrogenic effect can lead to an increase in the expression of the progesterone receptor.
- **Hormone production level:** α-ZOL, ZEN and β-ZOL modulate hormone production, especially progesterone production by β-ZOL.
- *In vivo* this may lead to combinatorial effects - since the three compounds affect the receptor level for this hormone.
- **Exposure** to ZEN and its metabolites via diet may have the potential to affect development and reproduction by binding to estrogen receptors and increasing levels of progesterone and its receptor.

Toxicon 90 (2014) 70–81

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Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Cytotoxic assessment of the regulated, co-existing mycotoxins aflatoxin B1, fumonisin B1 and ochratoxin, in single, binary and tertiary mixtures

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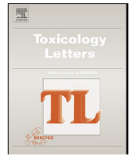

High content analysis: A sensitive tool to detect and quantify the cytotoxic, synergistic and antagonistic effects of chemical contaminants in foods

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Toxicology Letters 238 (2015) 54–64

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Toxicology Letters

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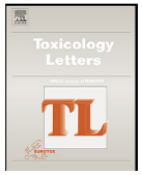

Challenging conventional risk assessment with respect to human exposure to multiple food contaminants in food: A case study using maize.

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Toxicology Letters

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Do persistent organic pollutants interact with the stress response? Individual compounds, and their mixtures, interaction with the glucocorticoid receptor

Jodie Wilson[a], Hanne Friis Berntsen[b], Karin Elisabeth Zimmer[b], Steven Verhaegen[b], Caroline Frizzell[a], Erik Ropstad[b], Lisa Connolly[a,*]

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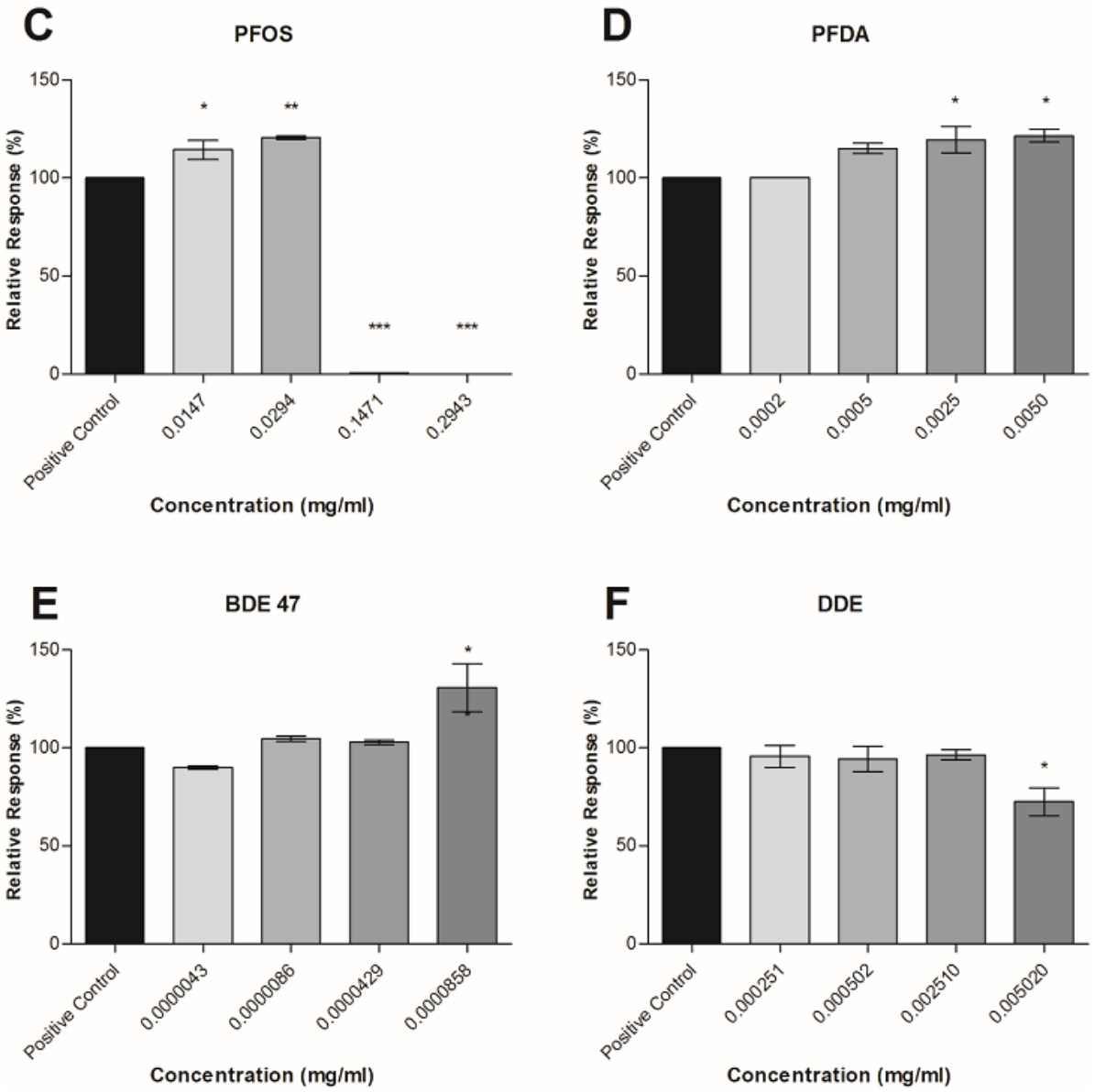
- Seven POP mixtures relevant to human exposure (Scandinavian serum levels) were screened for GR translocation using a GR redistribution assay on a high content analysis (HCA) instrument.
- In addition, 29 individual POPs, and the 7 mixtures, were assessed using a GR reporter gene assay (RGA).

Compound	Mixture Stock Concentration (mg/ml)							Individual Stock Concentration (mg/ml)
Perfluorinated compounds (PFCs)	**Total**	**PFC**	**Br**	**Cl**	**PFC+Br**	**PFC+Cl**	**Br+Cl**	
PFOA	4.523	45.225			45.225	4.523		45.225
PFOS	29.425	294.250			294.250	29.425		294.250
PFDA	0.495	4.950			4.950	0.495		4.950
PFNA	0.800	8.000			8.000	0.800		8.000
PFHxS	3.450	34.500			34.500	3.450		34.500
PFUnDA	0.560	5.600			5.600	0.560		5.600
Polybrominated diphenyl ethers (PBDEs)								
BDE-209	0.011		0.108		0.108		0.011	0.108
BDE-47	0.009		0.086		0.086		0.009	0.086
BDE-99	0.004		0.035		0.035		0.004	0.035
BDE-100	0.002		0.022		0.022		0.002	0.022
BDE-153	0.001		0.010		0.010		0.001	0.010
BDE-154	0.002		0.018		0.018		0.002	0.018
HBCD	0.025		0.246		0.246		0.025	0.246
Polychlorinated biphenyls (PCBs)								
PCB 138	0.222			0.222		0.222	0.222	2.220
PCB 153	0.362			0.362		0.362	0.362	3.620
PCB 101	0.008			0.008		0.008	0.008	0.078
PCB 180	0.194			0.194		0.194	0.194	1.940
PCB 52	0.010			0.010		0.010	0.010	0.096
PCB 28	0.013			0.013		0.013	0.013	0.128
PCB 118	0.064			0.064		0.064	0.064	0.640
Other organochlorines								
p,p'-DDE	0.502			0.502		0.502	0.502	5.020
HCB	0.117			0.117		0.117	0.117	1.170
α - chlordane	0.011			0.011		0.011	0.011	0.108
oxy - chlordane	0.022			0.022		0.022	0.022	0.222
trans-nonachlor	0.041			0.041		0.041	0.041	0.408
α-HCH	0.006			0.006		0.006	0.006	0.060
β-HCH	0.053			0.053		0.053	0.053	0.526
γ-HCH (Lindane)	0.006			0.006		0.006	0.006	0.060
Dieldrin	0.024			0.024		0.024	0.024	0.240



- No agonist effects or translocation on the GR were detected (mixtures or individual POPs).
- Significant increases in the RGA cell line transcriptional activity, in the presence of cortisol, was evident for PFOS, PFDA and BDE-47 – possibly suggesting GR receptor upregulation.
- The DDT metabolite p,p'-DDE reduced GR transcriptional activity to 72% (compared to the positive cortisol control).

Toxicology and Applied Pharmacology 294 (2016) 21–31

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Effects of defined mixtures of persistent organic pollutants (POPs) on multiple cellular responses in the human hepatocarcinoma cell line, HepG2, using high content analysis screening

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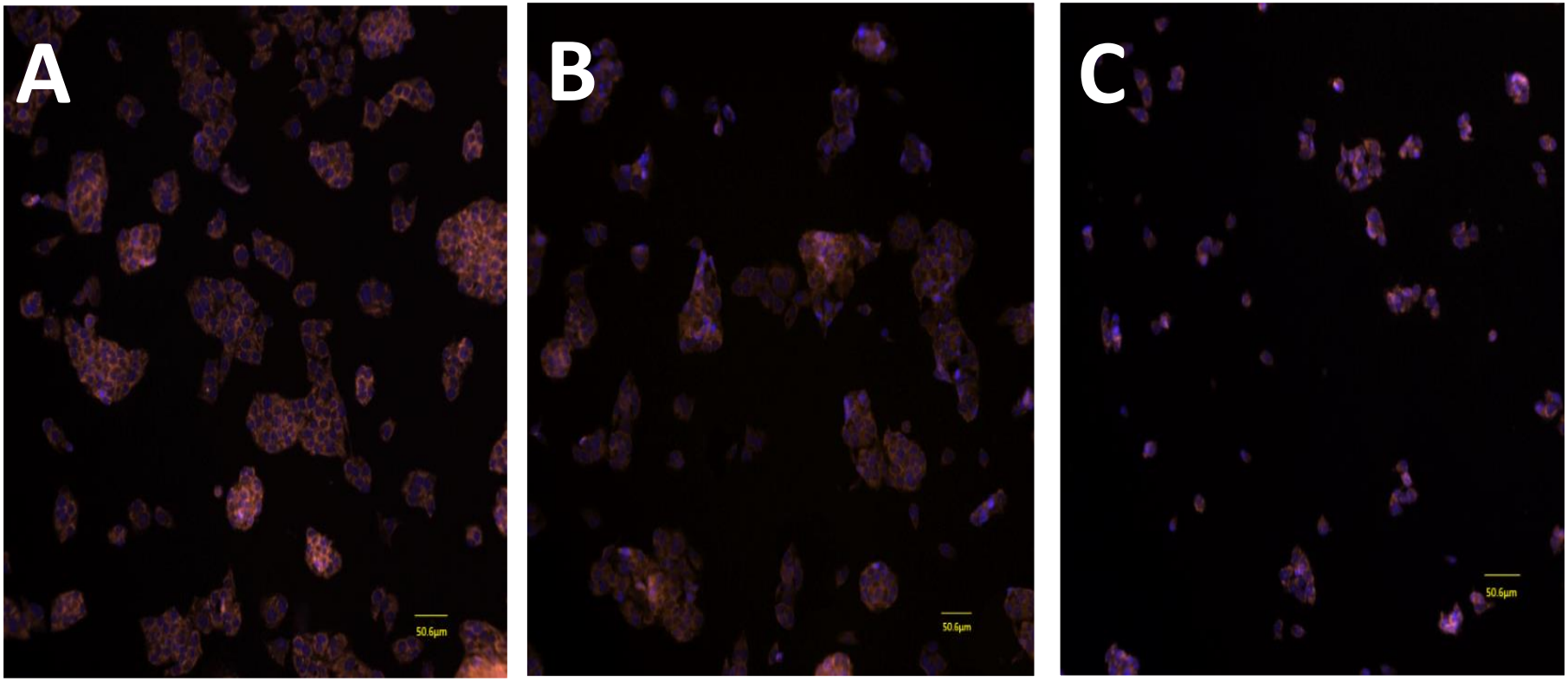
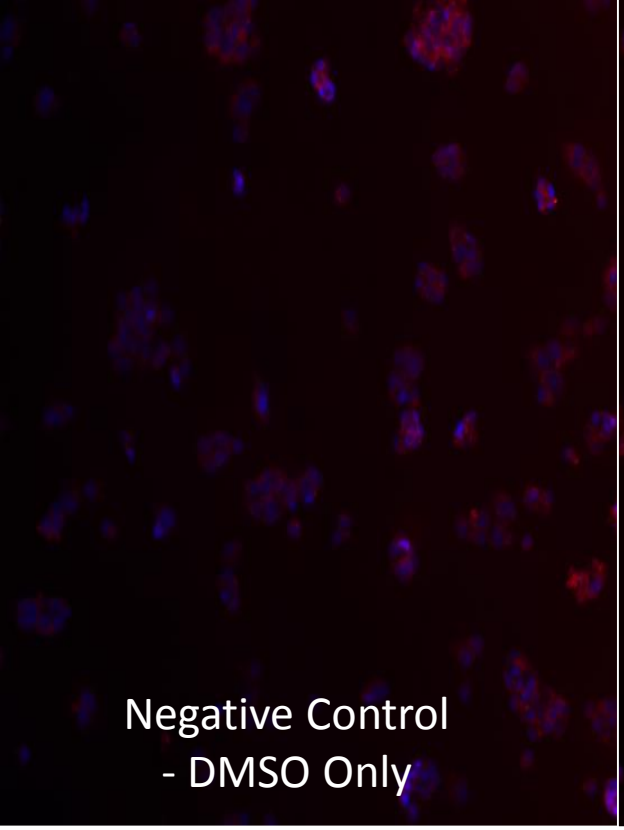
- 7 defined POP mixtures were investigated. HepG2 cells were exposed for 2 h and 48 h to detect early cellular events/effects and the eventual consequences thereof after 48 h.
- HCA cytotoxicity assay measured cellular parameters through the addition of dyes.

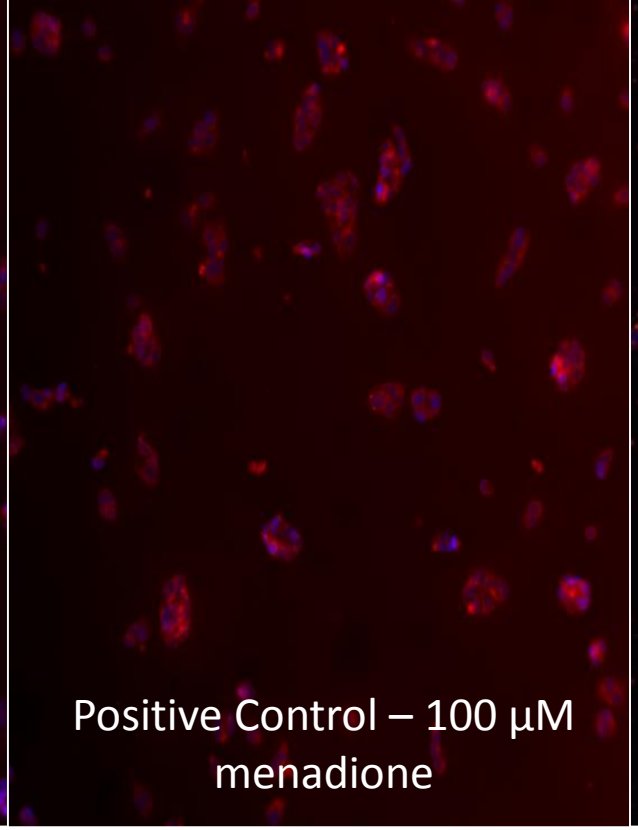
Dye	Measures
Hoechst stain (blue)	Cell number (CN), nuclear area (NA) and nuclear intensity (NI)
Mitochondrial membrane potential dye (orange)	Mitochondrial membrane potential (MMP) and mitochondrial mass (MM)
Cell ROX deep red reagent	Reactive oxygen species (ROS)

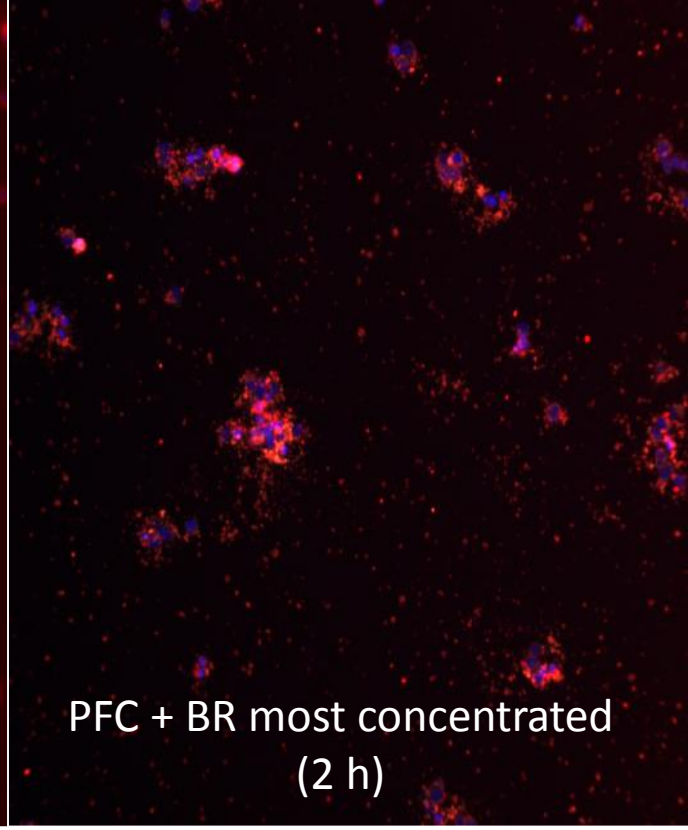
Mitochondrial Membrane Potential Dye (MMPD)



(A) negative control (DMSO), (B) positive control (100µM menadione), (C) example of mix - PFC + Br (10000 times serum level) – 2 h. Each image was acquired at 10 × objective magnification using Hoechst dye (blue; nuclear staining) and MMPD (orange; mitochondrial staining).







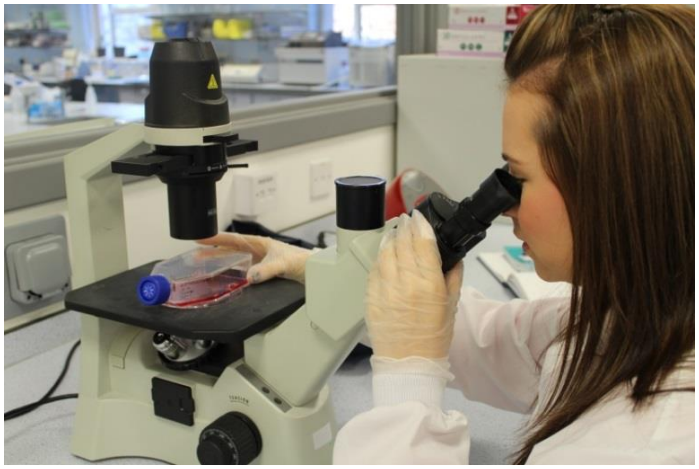
Example of CellROX deep red dye showing minimal red fluorescence in the DMSO control, increased fluorescence when exposed to the positive control and increased fluorescence in the presence of PFC + Br mix. The dye fluoresces when exposed to reactive oxygen species.

HCA cytotoxicity assay

POP mixtures can increase ROS induction and impact mitochondrial health, which could result in apoptosis.

- The mix of perfluorinated compounds (PFC mix) induced ROS production and likely induced cell apoptosis accompanied by the dissipation of MMP.
- The mix containing brominated compounds (Br mix) and the mix containing chlorinated compounds (Cl mix) induced ROS generation but it did not result in apoptosis.
- Furthermore a comparison of the PFC + Br mix to each independent mix showed that for some concentrations MM and ROS were synergistic.

Obesogens



Nutrients present in the gut

GLP-1 released into the bloodstream

GLP-1 binds to GLP-1 receptors on the surface of pancreatic beta cells

Appears to stimulate ***satiety*** by acting on GLP-1 receptors in the brain

Triggering ***release*** of insulin

Shannon Maeve, Rehfeld Anders, Frizzell C, Livingstone C, McGonagle C, Skakkebaek N, Wielogorska E, **Connolly L**. ***In vitro* bioassay investigations of the endocrine disrupting potential of steviol glycosides and their metabolite steviol, components of the natural sweetener *Stevia*.** *Molecular and Cellular Endocrinology*, accepted for publication March 2016.

Acknowledgements

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- Norwegian Research Council:
  - NFR grant 199412/199 "Mycotoxin contamination in Norwegian food and feed- Modelling, reductive approach and risk assessment with regards to the whole food chain".
  - NFR grant 21307/H10 “POPs and Stress”

Food safety, challenges present and future

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RSC Toxicology and Analytical Division, 8 March 2016

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The Food Standards Agency

to protect public health from risks which may arise in connection with the consumption of food, and otherwise to protect the interests of consumers in relation to food

- Putting the consumer first
- Openness and independence
- Science and evidence-based

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The Food Standards Agency

- Non-Ministerial Government Department
- Reports to Parliament and devolved administrations via Health Ministers, but not part of Department of Health
  - "Arms-length" from Ministers
- Headed by Chair and Board
- Wide powers to publish information and advice, including advice to Ministers guarantees independence

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Food safety challenges

- Microbiological
- Food allergy
- Chemical

- My personal perspectives

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- Challenges for risk assessors and risk managers
  - Financial influences
  - Climate change
  - Internet sales
  - Innovation in industry

- Challenges for the toxicologist
  - Developments in toxicity testing and risk assessment

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Financial issues

- Adulteration
- Authenticity

- Less resource for testing at import and on the market?

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Adulteration

European Food Safety Authority

The EFSA Journal (2008) 807, 1-10

STATEMENT OF EFSA

Statement of EFSA on risks for public health due to the presences of melamine in infant milk and other milk products in China

(Question No. EFSA-Q-2008- 695)

Issued on 24 September 2008

Summary

The European Food Safety Authority (EFSA) received a request on 19 September 2008 from the European Commission, Health and Consumers Directorate requesting

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Adulteration

Businesswoman sold 'edible' cupcake glitter made from shredded plastic and powdered brass - and insisted it was safe despite repeated warnings

- Margaret Martin duped cake businesses into buying the fake 'edible' glitter
- Despite being marketed as edible decoration, it was made from plastic
- Martin claimed she named her business EdAble Art Ltd after three animated mice characters called Ed, Able and Art
- Leeds Magistrates' Court ordered her to pay £13,515 in costs and fines

By EMMA GLANFIELD

PUBLISHED: 17:21, 13 March 2014 | **UPDATED:** 21:20, 13 March 2014

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Adulteration

One in four dried oregano samples contains other ingredients

Food fraud study reveals 19 out of 78 samples of herb from UK and Ireland retailers had 30% to 70% other ingredients, such as olive and myrtle leaves

Handout photo issued by Which? of olive leaves (left) and oregano leaves (right), as a study has found that a quarter of samples of dried oregano contained other ingredients, most commonly olive and myrtle leaves. Photograph: Which?/PA

A quarter of samples of dried oregano have been found to contain other ingredients in the latest food fraud

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Adulteration

Fake rice made with plastic reportedly spreading across Asia

Plastic rice sold on the Chinese market has reportedly found its way into various Asian countries, including India, Indonesia and Vietnam.

The fake rice is made by mixing potatoes, sweet potatoes and synthetic resin, according to the International Business Times. It's long been circulating on the market in Taiyuan, Shaanxi and appears identical to natural rice.

Health experts have warned that consuming the fake grains could seriously damage the digestive system.

Authorities have attempted to quell consumers' fears as the internet has been abuzz with news of the fake rice landing on the shores of various Asian regions.

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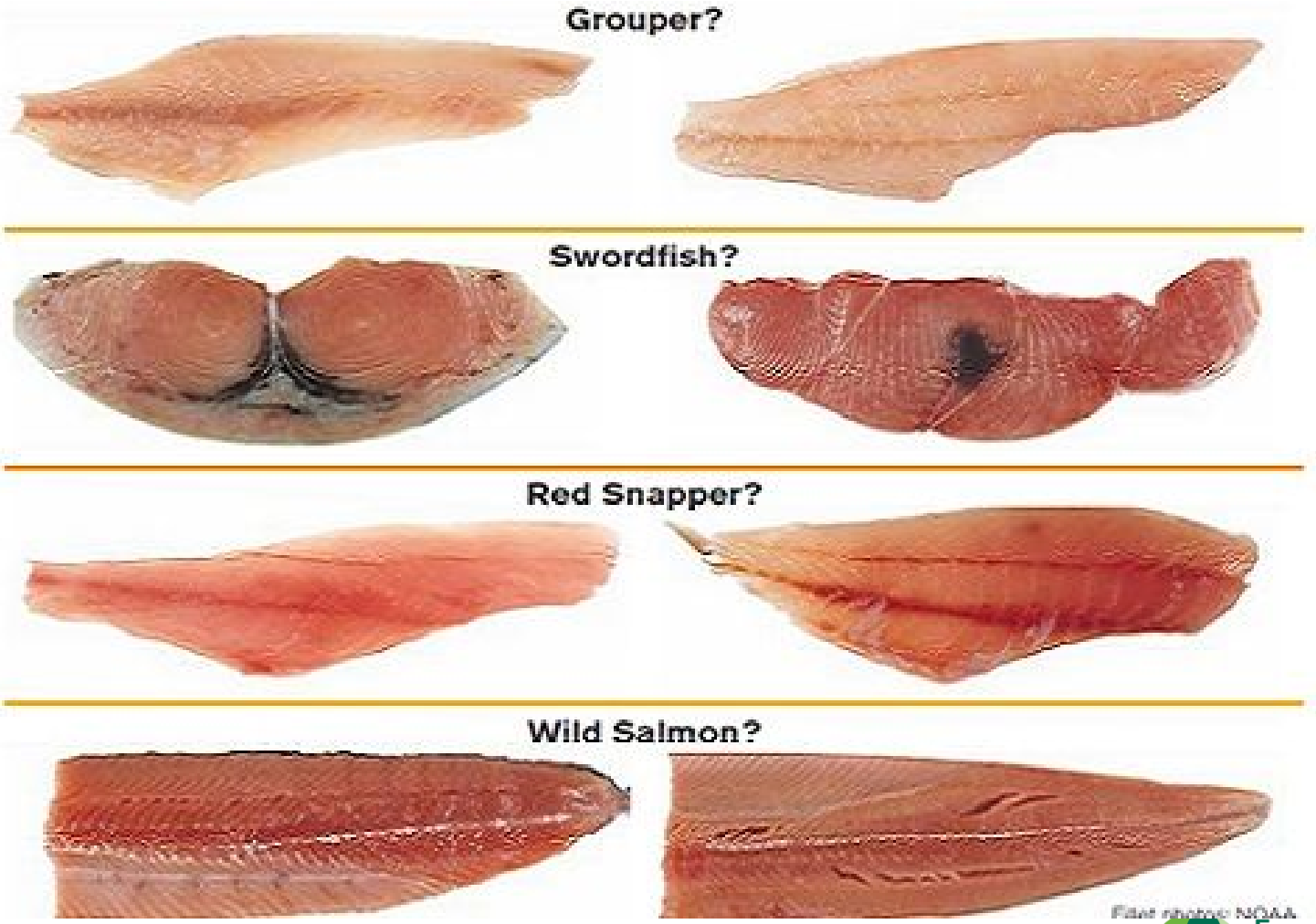
Authenticity



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14 February 2013 Last updated at 17:16

Share

Horsemeat scandal: Bute found in eight horse carcasses

Eight horses, killed in the UK, tested positive for the painkiller bute and six may have entered the food chain in France, the Food Standards Agency said.

England's chief medical officer said the highest level detected was 1.9mg of bute per

EFSA Reference Points for Action

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What is next?

How do we target limited resources for monitoring?

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Possible impacts of climate change

- Mycotoxins
- Marine biotoxins
- Impact on agricultural practice
- Flooding

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Regulated mycotoxins - 1

- Aflatoxins
  - Field and storage moulds, especially in hot/humid climates
  - Genotoxic carcinogens
  - Margins of exposure are small
  - Frequent non-compliant commodities at import
  - Future problem within the EU?



Regulated mycotoxins - 2

- Increasing non-compliance
  - Deoxynivalenol (DON)
  - Zearalenone
  - Fumonisins
  - Grow on cereal crops in EU
- Occasional non-compliance
  - Ochratoxin A
  - Mainly due to storage conditions

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Regulated mycotoxins - 3

- Patulin
  - Primarily on mouldy apples
- Citrinin
  - Stored grains
  - ML applied to red rice

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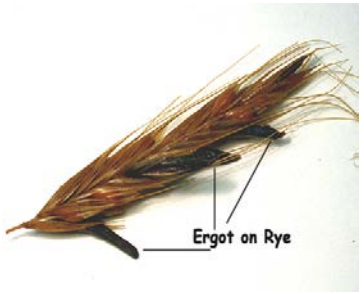
Partially regulated

- Ergot alkaloids
  - Regulation currently based on sclerotia



- Nivalenol?
  - Co-occurs with DON
  - Regulation so far not needed

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Non-regulated mycotoxins

- Sterigmatocystin
- Phomopsins
- T2/HT2
- Alternariol
- Beauvericin
- Enniatins
- Moniliformin
- Diacetoxyscirpenol
- Others?

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Mycotoxin challenges

- Multiple related toxins
- Lack of toxicity data for some toxins
- Lack of data on relative potency for some toxins
- Will exposure increase with climate change
- Are analytical methods available for all relevant toxins
- Are all relevant toxins included in the regulations?

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Regulated marine biotoxins

- okadaic acid group
- azaspiricids
- yessotoxins
- saxitoxins
- pectenotoxins
- domoic acid

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Non-regulated biotoxins

- Cyclic imines
- Palytoxins
- Brevetoxins
- Ciguatoxin
- Tetrodotroxin
- Other?

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Biotoxins challenges - chemistry

- Pure toxins are available in very low amounts
- Not all relevant toxins have been purified
- Have all relevant toxins been identified?
- Are the analytical methods adequate?

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Biotoxins challenges - toxicity

- Acute toxicity data in laboratory animals
  - Mostly in mouse with intra-peritoneal injection
  - Some oral data for some toxins
- Very few if any repeated dose toxicity studies in animals
- Lack of data on relative potency
- What are the combined effects of different
  classes of toxins that co-occur?

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Biotoxins - future challenges

- Possible changes in exposure to marine biotoxins due to:
  - Climate change?
  - Human activity, such as shipping?
  - Evolving species?





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Agriculture practice

- Changes in crop production
  - Altered pesticide usage and exposure?
- Changes in diseases of livestock
  - Altered usage of veterinary medicines?
- Contamination by toxin-containing weed species?
  - Tropane alkaloids
  - Pyrollizidine alkaloids

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Pyrrolizidine alkaloids (PAs)

- PAs are biosynthesised by plants as secondary metabolites against herbivores.
- Approximately 6000 plant species world wide may contain PAs.
- 95% of PAs are found in 5 plant families: Asteraceae (Compositae), Boraginaceae, Fabaceae (Leguminosae), Orchidaceae and Apocynaceae.
- > 600 known PAs




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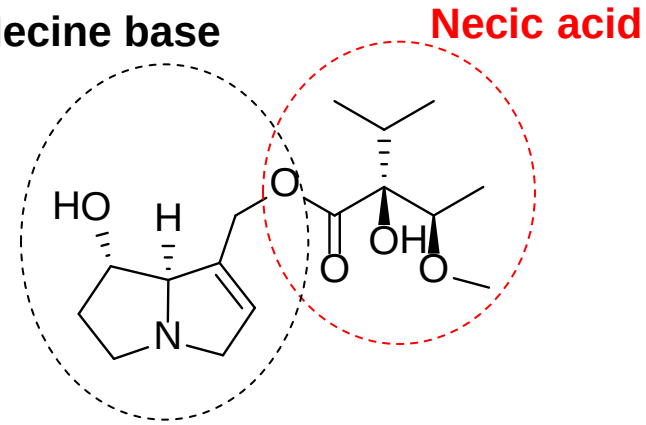
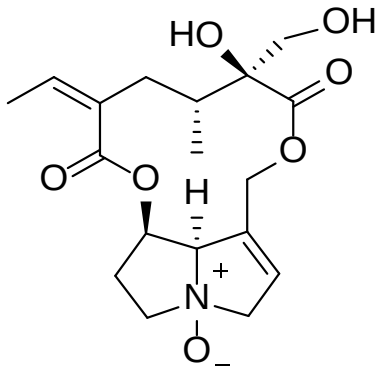
Chemical structures of PAs

- 600 known PAs, as a result of combinations of different necine bases and necic acids, as mono-, di- and cyclic esters.
- Necine bases can be 1,2-saturated or 1,2-unsaturated.
- Very little information is available on saturated PAs
- Many PAs co-occur in 2 forms: PA and corresponding PA-N-oxide (PANO).



Example of PA, heliotrine



Example of PANO, retrorsine-*N*-oxide

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Potential sources of PAs in food

- Honey
  - If bees harvest pollen of PA-plants
- Pollen dietary supplements
- Salad crops
  - contaminated with PA-plants such as *Senecio vulgaris*
- Herbal products, supplements and teas
  - Prepared from, or contaminated by, PA-containing plants
- Products of animal origin (meat/milk/eggs)
  - If food producing-animals graze on PA-plants or feed contaminated with them
- Cereals
  - If grain crops are contaminated with PA-plants





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Most common PAs in food

- Senecionine-type PAs: acetylerucifoline, erucifoline, integerrimine, jacobine, jacoline, jaconine, jacozine, retrorsine, senecionine, seneciphylline (Asteraceae and Fabaceae families).
- Lycopsamine-type PAs: acetylechimidine and isomers, echimidine and isomers, echivulgarine, lycopsamine and isomers, vulgarine (Boraginaceae and Asteraceae).
- Heliotrine-type PAs: europine, heliotrine, lasiocarpine (Boraginaceae).
- Monocrotaline-type PAs: fulvine, monocrotaline, retusamine, trichodesmine (Fabaceae).

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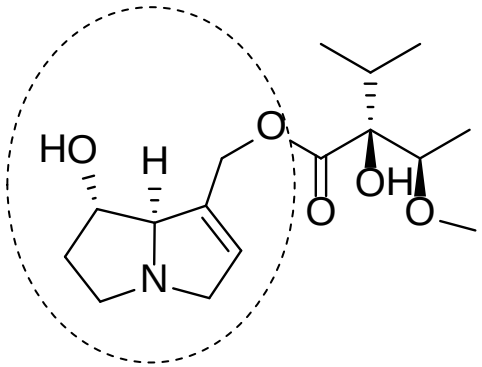
Challenges with PAs - chemistry

- Which are the most relevant PAs?
- Limited availability of reference material/standards
- Is the methodology adequate?
- Measurement of individual PAs, or combination



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Challenges with PAs - toxicity

- Of the hundreds of PAs identified in PA-containing plants, relatively few have been measured in food, and analyses have focussed on a small number of food types. Therefore dietary exposure estimates are not comprehensive
- 1,2-unsaturated PAs are genotoxic and carcinogenic. Very little is known about the toxicological properties of 1,2-saturated PAs.
- Based on the common mode of action the effects of 1,2-unsaturated PAs are likely to be additive, but the available data are not sufficient to identify relative potency factors
- Herbal dietary supplements have the potential to result in the highest exposure and have been known to cause acute human illness
- Could climate change increase exposure?

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Possible impact of flooding

- Redistribution of residues from rivers
  - Heavy metals
  - Persistent organic pollutants

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Internet sales

- Quality and safety cannot be assured
- Alternative therapies, e.g.
  - Herbals
  - Fat burner supplements
  - Miracle mineral supplement (MMS)
  - Bitter apricot kernels

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Herbals

- Common perception that “natural” is healthy, synthetic is bad
- Regulation under general food law – must not be injurious to health
- History of safe use is claimed, but can it ever be established?
- Preparations poorly characterised

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Fat burners

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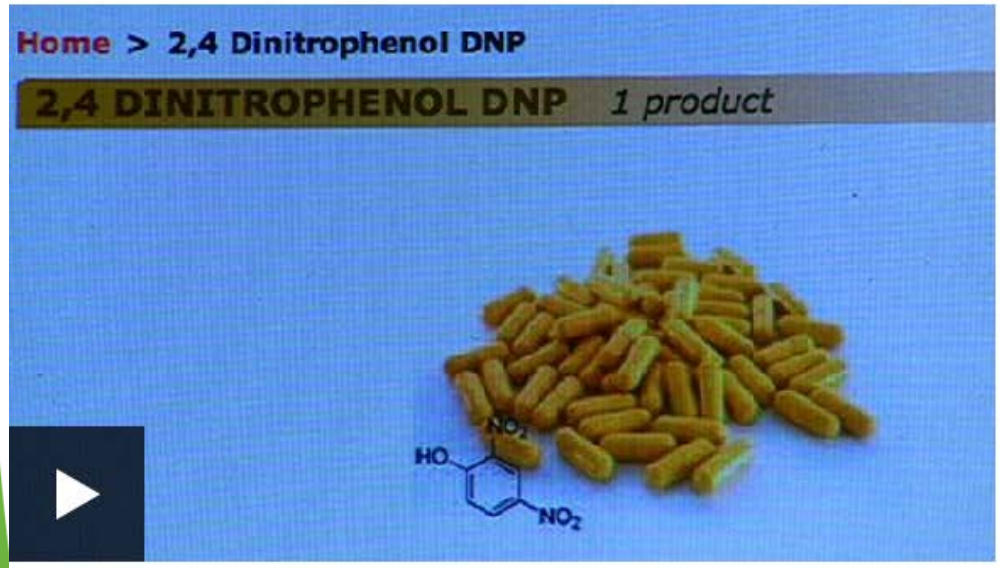

Warning over illegal 'weight loss' chemical DNP

9 October 2013 Last updated at 08:17 BST

The Food Standards Agency is launching a campaign to warn people of

Related video / audio

Parents urge ban on slim drug

19 September 2013

FBI shuts down Silk Road website

3 October 2013

Exercise 'can be as good as pills'

2 October 2013

Counterfeit drugs 'may kill you'

26 September 2013

Most watched

Miracle mineral solution

NEWS HEALTH

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24 September 2010 Last updated at 13:10

Alert issued on danger supplement

Food watchdogs have issued an alert after finding that a chemical marketed online as a health supplement was similar to industrial-strength bleach.

'Miracle Mineral Supplement' is 28% sodium chlorite - which becomes bleach when mixed with citric acid.

Even taken as instructed, experts say it can cause severe vomiting and diarrhoea - and mixing it wrongly could lead to respiratory failure.

The Food Standards Agency is seeking to track down any UK-based sellers.

The supplement, which claims to have beneficial effects against a wide range of illnesses, ranging from HIV and hepatitis to H1N1 flu, has already been the subject of warnings from the Food and Drug Agency (FDA) in the US.

Now UK consumers are being urged to dispose of the supplement as soon as possible, and help the Food Standards Agency and local authorities find suppliers in this country.

It is still apparently on sale here through a number of sites, one offering a 100ml bottle for £33.

A spokesman for the Food Standards Agency said: "People should not take Miracle Mineral Supplement or Miracle Mineral Solution.

"This product is equivalent to industrial-strength bleach.

"The agency is working with local authorities across the UK to stop this product being sold. If anyone finds this product on sale please get in touch with your local authority trading standards department as soon as possible."

"This product is equivalent to industrial-strength bleach"

Food Standards Agency spokesman

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Bitter apricot kernels

- Marketed as anticancer agent or as component of a “natural healthy diet”
- Contain high levels of cyanogenic glycosides
- Conspiracy theorists do not accept official advice

Emerging issues

- Technological advances in food production
- New releases to the environment
- New approaches to risk assessment
- Mixtures

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Innovation in the food industry

- For example
  - Insects
  - Nanomaterials
  - Intelligent packaging
  - 3D printing
  - Synthetic biology
- Commercial sensitivities restrict availability of information to support risk assessment
- Need to consider potential for transfer of contaminants to the environment as well as direct risks
- Challenges for analytical methodology?

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New industrial chemicals

- Example – brominated flame retardants (BFRs)
- PBDEs phased out due to concern about persistence in the environment
- EFSA
  - 17 ‘emerging’ BFRs that have been identified in wildlife, food or humans
  - 10 ‘novel’ BFRs – known applications but not identified in the environment
  - Potential for some to be persistent and bioaccumulative
  - Insufficient information on toxicity and exposure for risk assessment

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Challenges to the toxicologist

- Increasing detection of multiple chemicals in food and the human body
- Insufficient toxicity data on many chemicals
- Limited amount of material for testing of some substances, such as individual toxins
- Insufficient resource for testing by conventional methods
- Need data to support cumulative risk assessment approaches
- Need risk ranking methodology to prioritise areas for testing

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Advances in toxicity testing and risk assessment

- Developing areas
  - What is adverse
  - Uncertainty analysis

- New approaches in toxicity assessment
  - Omics
  - Tox21
  - Adverse outcome pathways

  Uses in screening, determining human relevance, prioritisation for further testing

  When will we have enough information to be confident of their use in risk assessment?

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Mixtures

- Exclusion approach for pesticides
- Inclusion approach for other chemicals
  - An exclusion approach is not feasible for the “world of chemicals”
- Current approaches require extensive datasets
- Need approaches to prioritisation and predictive modelling

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The future

- Food defence
- Concern about food waste

- Impact of the microbiome on individual sensitivity
- “Big data”
- Social media
- Artificial intelligence

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Summary

- We have to expect more instances of food adulteration and fraud. We need to determine whether there are risks to the consumer, frequently with limited information
- Climate change has the potential to alter dietary exposure to toxins. Toxin groups often comprise multiple chemicals, with limited data available to assess toxicity and to support cumulative risk assessment
- The safety and quality of substances available on the internet often cannot be assured
- Innovation in the food industry and other sectors has the potential to introduce new contaminants into the food chain

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Conclusion

- Risk assessment is required for increasing numbers of chemical substances and combinations thereof, frequently based on limited data
- Analytical chemistry is an essential component to ensure we have data on the most relevant substances and that they are reliable
- We need new approaches to food surveillance

What happens if we leave the EU?

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Thank you

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