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8th BioDetectors 2014

Applications of bioassays to prioritize chemical food safety issues

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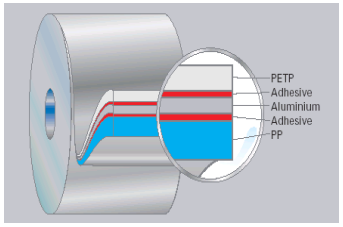
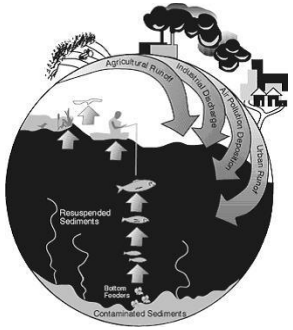
Hazard identification in food industry



substances potentially used in packaging industry



(adapted from U.S. EPA)

PRESENT IN FOOD

substances potentially coming from environmental, agricultural practices and process-related contamination

Diverse sources of potential food contamination

Hazard identification: Which are the tools available?

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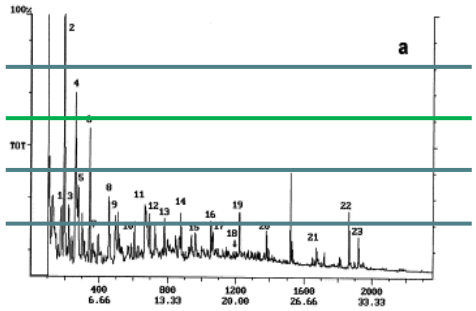
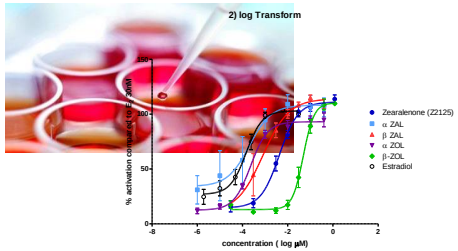
- **Chemistry**
- **Toxicology**



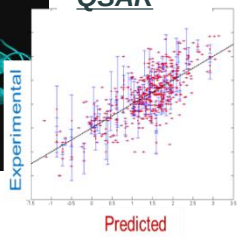
In vitro



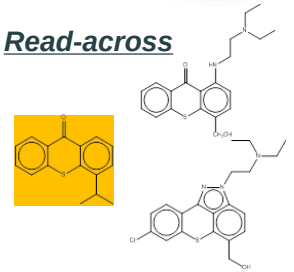
In vivo

Docking

QSAR



Read-across



Analytics

Bioassays

In silico

Knowing the sources: e.g. dealing with food packaging

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- Expected/known
  - Intentionally Added Substances **IAS**
  - Regulatory toxicology data
  - Safe level of exposure

Risk Assessment principles

Compliance

- Unexpected/unknown
  - Non-Intentionally Added Substances **NIAS:**
    - Impurities in ingredients
    - Reaction intermediates/products
    - Decomposition products
  - Many?
  - Not characterized?

Food packaging safety evaluation

- No harmonized & standardized procedures
- Traditional toxicology-based methodology requiring full characterization of all substances neither practical nor feasible

Multidisciplinary approach for packaging safety

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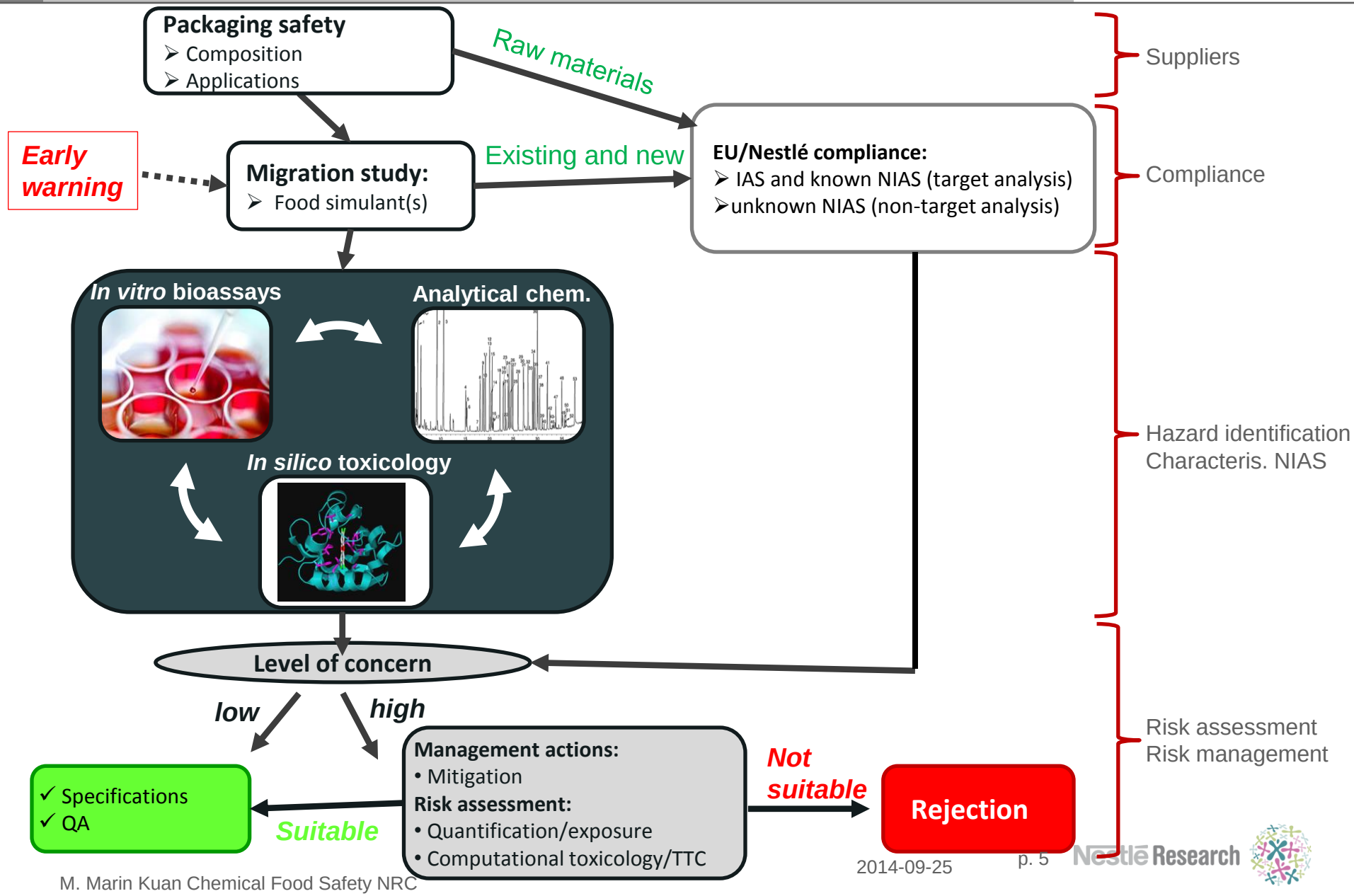

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In vitro tools for the "biodetection" of potential food active compounds

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I. Sound Biological Targets in toxicology

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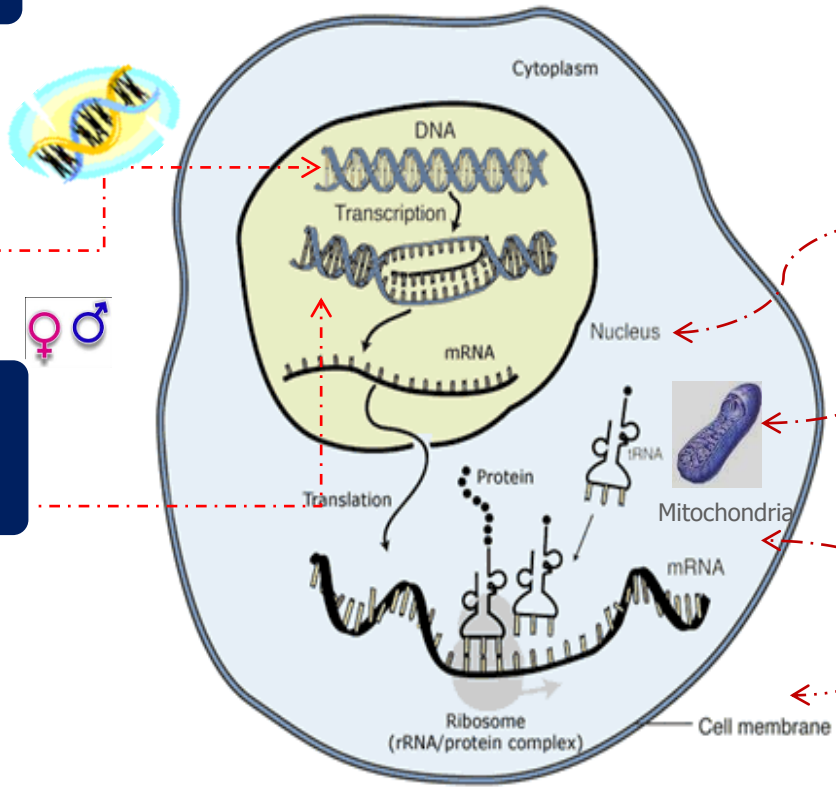
Reactive toxicity

GENOTOXICITY
GADD45α
H2AX
p53

Effect-based toxicity

ENDOCRINE ACTIVITY
ESTROGEN
ANTI-ESTROGEN
ANDROGEN
ANTI-ANDROGEN
THYROID
PPAR



Non-specific toxicity

CYTOTOXICITY

CELL LOSS/ DNA CONTENT

MITOCHONDRIAL MEMBRANE POTENTIAL

CYTOCHROME C

CELL PERMEABILITY

Image adapted from:
National Human Genome Research Institute

II. Nestlé BioDetection battery of tests

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Endpoints	Platform/method	Bioassays
Endocrine activity	ToxInsight	Estrogen & androgen receptor redistribution assays
	CALUX	**Estrogen, androgen, AhR, thyroid, PPARγ2 receptor activation**
	ELISA	H295R Steroidogenesis assay (hormone production)
Genotoxicity	ToxInsight	**Histone γH2Ax phophorylation**
	ToxInsight	Micronucleus assay
	Bluescreen	**Gadd45α expression**, Ames test (mutagenicity)
	p53	
Cytotoxicity	ToxInsight	Organelle health (mitochondrial markers)
		Cell proliferation (DNA, Br-deoxyuridine incorporation) and cell death (apoptosis, caspase 3, p53)
	Xenometrix	**Cytotoxicity screening**: protein synthesis, lysosomial and nuclear membrane integrity biomarkers

- Speed, convenience, high content, robustness, relevance
- Ability to cover several mechanisms/modes of action

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Case studies: Application of the biodetection tool battery

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Case study 1: plastic material in development Identifying responsible compounds

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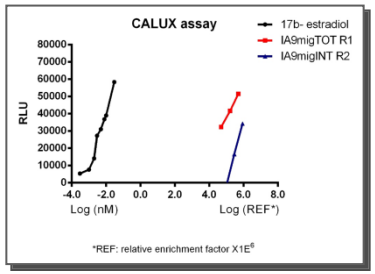
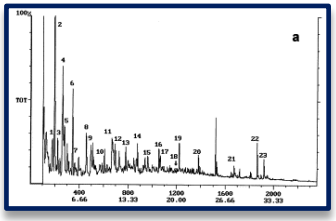
Material bioactivity:
- **Migration simulation assay**
- **In vitro battery:**
  - ***toxicity***
  - ***endocrine***
  - ***genotoxicity***

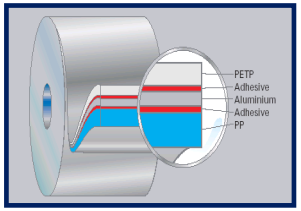


Estrogenic activity

Analytical profile



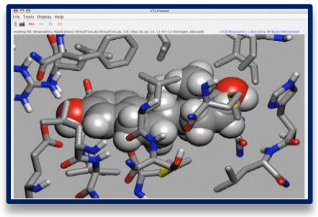
Information on material: ingredients, recipes



Verify *in vitro*:
- *effect(s)*
- *dose-response*

Comparison with analytical data*:*
- *concordance*

List substances

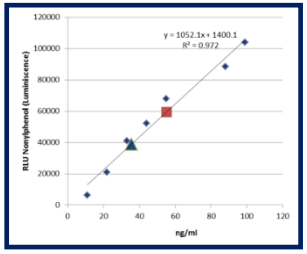


substance with known endocrine activity:

4-nonylphenol

***In silico* screen for alerts**

Adapted CALUX test



⇒ Confirmation *in vitro*

Case study 2: presence of azaarenes in food
Comparing toxicity to known analogues

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- **Case study**:

2858

J. Sep. Sci. 2012, 35, 2858–2865

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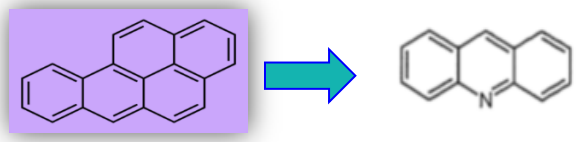
Research Article

Determination of azaarenes in oils using the LC-APCI-MS/MS technique: New environmental toxicant in food oils

- **Background**: PAH-nitro derivatives:



  - Structural and sources similarities with PAHs but less well characterized
  - Very limited information on levels in food and exposure (e.g. oils)

- **Toxicological data:**
  - » Limited toxicological data
  - » Evidences suggest stronger toxicity as compare to PAH
  - » **Further information is needed to define the toxicity of PANHs as compared to PAHs**

Known PAHs mode of action

➔Reference compound: Benzo(a)pyrene

- *Genotoxic after metabolic activation: Protein and DNA-adducts formation*
- *Potent Aryl hydrocarbon Receptor (AhR) activator*
- *Other nuclear receptors also involved (e.g. ER)*

Tested Compounds

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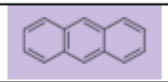
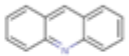
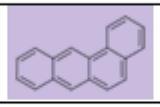
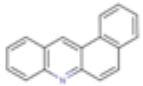
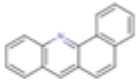
CONTROL compound

3 rings

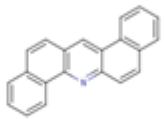
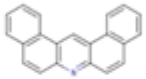
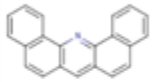
3 rings	Anthracene	Acridine
	Anth	Acr
Structure		
N° CAS	120-12-7	260-94-6
Molecular Mass (g/mol)	178.23	179.22
Log Kow	4.45	3.40
Stock Concentration (mM)	50	500

5 rings	Benzo(a)pyrene
	BaP
Structure	
N° CAS	50-32-8
Molecular Mass (g/mol)	252.3
Log Kow	6.00
Stock Concentration (mM)	50

4 rings

4 rings	Benz(a)anthracene	Benz(a)acridine	Benz(c)acridine
	BaAnth	BaAcr	BcAcr
Structure			
N° CAS	56-55-3	225-11-6	225-51-4
Molecular Mass (g/mol)	229.29	229.29	229.28
Log Kow	5.79	4.48	4.61
Stock Concentration (mM)	100	200	200

5 rings

5 rings	Dibenz(a,h)anthracene	Dibenz(a,h)acridine	Dibenz(a,j)acridine	Dibenz(c,h)acridine
	DiBahAnth	DiBahAcr	DiBajAcr	DiBchAcr
Structure				
N° CAS	53-70-3	226-36-8	224-42-0	224-53-3
Molecular Mass (g/mol)	278.35	279.35	279.35	279.35
Log Kow	6.75	5.73	5.63	6.45
Stock Concentration (mM)	12.5	10	50	50

Data summary

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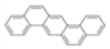
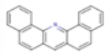
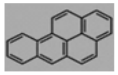
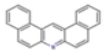
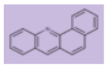
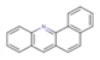
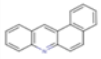
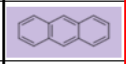
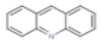
In vitro bioassays summary (μM)

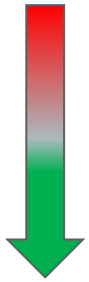
Sample				Nuclear receptors					Genotoxicity				Cytotoxicity	
Type	Rings	Compound	Structure	AhR	Er α	Anti ER	AR	Anti AR	Gadd45a (+S9)	Gadd45a (-S9)	H2AX	p53	(+S9)	(-S9)
PANH	5R	Dibenz(ah)acridine		0.0004	-	16.58	-	-	80	-	5.0		-	-
		Dibenz(ch)acridine		0.001	-	-	-	1.19	-	-	1.6	17	-	118
PAH		BaP		0.002	0.97	-	-	0.63	12.5	-	0.1	10	11	-
PANH		Dibenz(aj)acridine		0.0025	-	-	-	9.56	-	-	-	50	-	41
PAH		Dibenz(ah)anthracene		0.01	-	-	-	49.00	-	-	0.1	33	-	-
PAH	4R	Benz(a)anthracene		0.002	3.03	-	-	0.22	-	-	100	33	-	-
PANH		Benz(c)acridine		0.20	35.00	-	-	0.82	-	800			257	-
		Benz(a)acridine		1.42	23.00	-	-	1.91	200	400	200	200	31	203
PAH	3R	Anthracene		-	15.50	-	-	0.97	-	-			-	-
PANH		Acridine		-	142.00	-	-	3.40	-	400		167	83	-

EC_{50}

LPC
(lowest (+) concentration)

$CD_{40\%}$

Weak activator



Stronger activator

General summary

- **Reference compound: BaP**
  - Results in line with the literature: cytotoxic, genotoxic and nuclear receptors activator: AhR, ERα and anti-AR
  - Confirm the role of metabolic activation
  - Battery of bioassays works appropriately

- Receptor-mediated effect
  - AhR: Most sensitive parameter (2-3 orders of magnitude)
  - Correlation between AhR activity and number of rings
  - Decreased activation of ERα receptor with the number of rings (less potent AhR)
  - Anti-AR effect with all compounds (except Dibenz(ch)acridine)

- Other parameters
  - No correlation between AhR activation and other parameters
  - No consistent trends between PAHs vs PANHs regarding cytotoxicity and genotoxicity potency
  - Some evidences of higher genotoxicity and cytotoxicity induction by the 5-rings PAHs and its derivatives
  - No good consistency across genotoxicity tests

Conclusions case study 2

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- Compared to PAHs, azaarenes are likely to have similar toxicological profile
- However, they may not bring additional risk burden since exposure seems much lower than for PAHs

Cell culture-based *in vitro* battery summary

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- Receptor-mediated effect model: CALUX model is a sensitive test for receptor activation studies
  - Need to address the role of metabolic activation on nuclear receptors activation parameter

- Genotoxicity

  Optimization of the genotoxicity assessment is needed to address consistently:
  - sensitivity
  - specificity
  - metabolic activation
  - optimal cell model

- Data interpretation for decision making: cut-off values and/or trigger values needed

- *In vitro* toxicology issues for decision making, need to be address through a multidisciplinary approach

Acknowledgements

- **Chemical Food Safety Group Nestlé Research Center:**
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  - Claudine Bezençon

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THANK YOU FOR YOUR ATTENTION

Questions
or
Comments ?