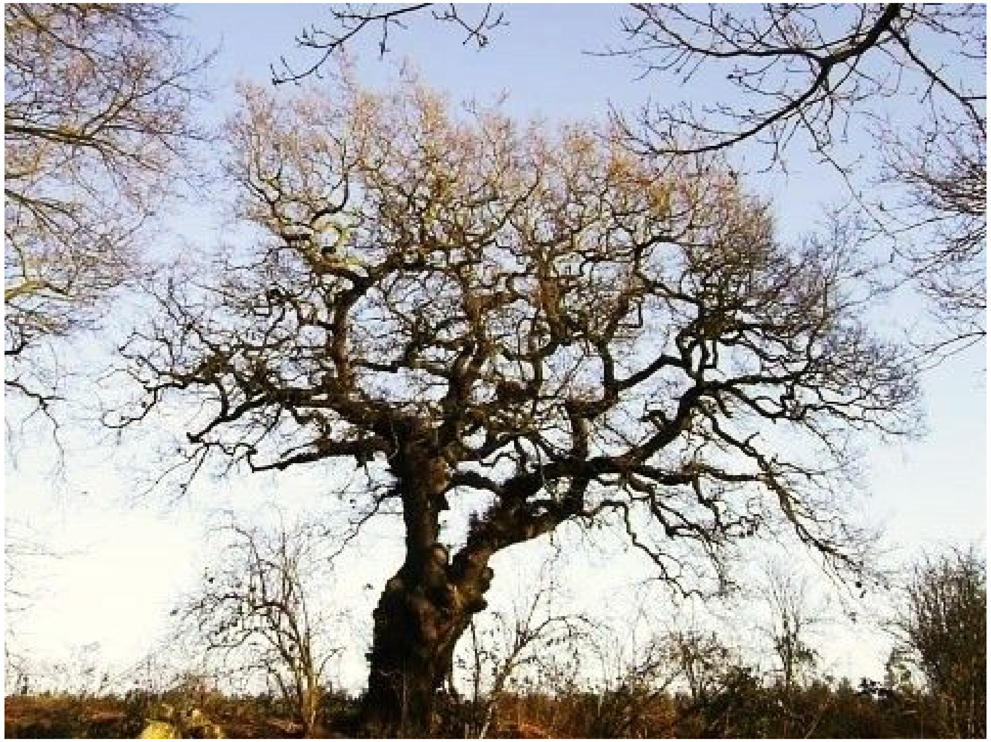
Cramer Decision Tree (DT)
Threshold of Toxicologic Concern (TTC)



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The Cramer/Ford/Hall Decision Tree

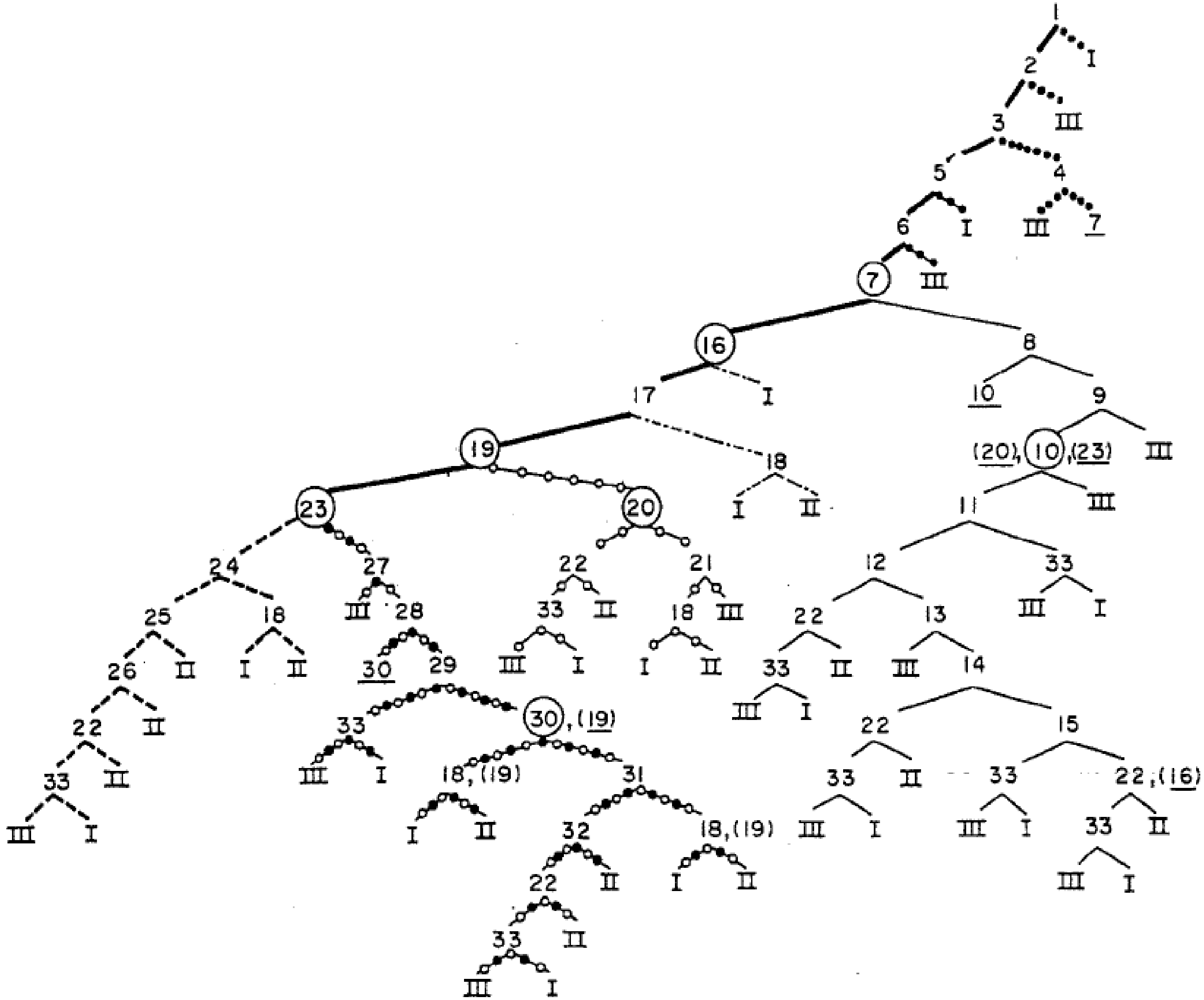
- Published in 1978
  - *Food Cosmet. Toxicol.* (1978) **16**, 255-276
- Relates chemical structure to toxic potential
- Screening for toxicity testing
- Validated using toxicity and metabolism data for pesticides, drugs, food additives, industrial chemicals, flavors, fragrances
  - Updated in 1996 (*Munro, et al.,1996*)

Decision Tree –Structural Classes

- Class I
  - Structures that suggest a low order of oral toxicity. If low human exposure, then a low priority for further testing (e.g., ethyl butyrate)
- Class II
  - Less clearly innocuous than Class I, but no firm indication of toxicity or the lack thereof (e.g., furfural)
- Class III
  - Structure permit no initial presumptions of safety, or may suggest toxicity. - highest priority (e.g., anethole)

The Decision Tree Structure

- biological normality
- high and low toxicity
- heterocyclics
- terpenoids
- aromatics
- aliphatics
- alicyclics



Threshold of Toxicologic Concern (TTC)

- Munro *et al.* 1996
  - Collect toxicity data for each DT Class: I, II, and III
- Organize NOELs by Structural Class
- Identify 5^{th} % NOEL for each Class (Class I, 3.0 mg/kg/d
- Assume 100-fold safety factor
- Define TTC for each DT Class:
  - 5% NOEL(µg/kg/d) x 60 (kg/p) x 1/100=TTC (µg/p/d)

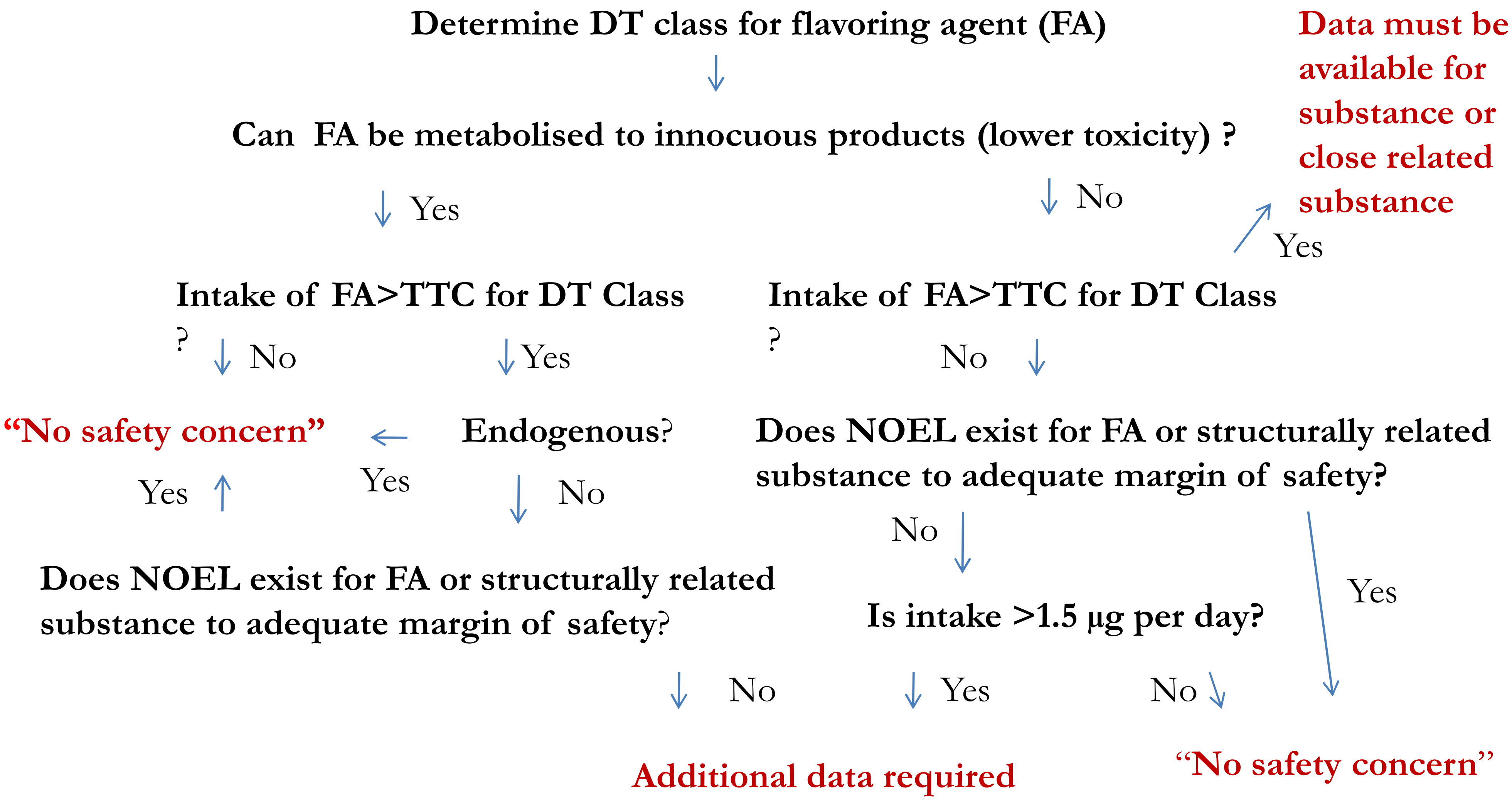
Relation of DT Class to TTC (Munro, 1996)

Structural Class (examples)	5th% NOEL, mg/kg/d	TTC (ug/p/d
I (ethyl butyrate, cinnamaldehyde)	**3.0**	**1800**
II (3,6-dimethylpyrazine, pulegone)	**0.91**	**544**
III (estragole, anethole)	**0.15**	**90**

Application of DT and TTC in Safety Evaluation (1996-present)

- International
  - Codex/JECFA 1995 evaluation procedure
    - Use DT and TTC
- Regional
  - European Food Safety Authority 2000 (EFSA)
    - Adopt JECFA procedure
  - FDA, FEMA GRAS
    - Use as screening method-1978

Use of TTC and DT in JECFA Procedure, 1996



How the JECFA Procedure Works

DT class for benzaldehyde I

↓

Metabolism to glycine conjugate of benzoic acid -innocuous

↓ **Yes**

↓ No

Data must be available for substance or close related substance

Yes

Intake, 52,000 µg per day > 1800 µg per day

Intake of FA>TTC for DT Class ?

↓ No

↓ **Yes**

No ↓

“**No safety concern**” ← **Endogenous**?

Does NOEL exist for FA or structurally related substance to adequate margin of safety?

Yes ↑

Yes

↓ **No**

No ↓

Yes

NOEL of 200mg/kg bw/d [NTP, 1990] is >100 times the daily per capita intakes ("eaters only").

Is intake >1.5 µg per day?

↓ No

↓ Yes

No ↓

Additional data required

“No safety concern”

How the JECFA Procedure Works

DT of 2-pentylfuran III

↓

May be bioactivated by furan ring oxidation yielding reactive "enonal"

↓ Yes

↓ **No**

Data must be available for substance or close related substance

↗ Yes

Intake of FA>TTC for DT Class ?

Intake, 0.2 µg per day < 90 µg per day

↓ No

↓ Yes

No ↓

"No safety concern"

← Yes

Endogenous?

NOEL of 25.6 mg/kg bw per day for 2-pentylfuran (Shellenberger, 1971) is 6,400,000

Yes ↑

↓ No

No ↓

Yes

Does NOEL exist for FA or structurally related substance to adequate margin of safety?

Is intake >1.5 µg per day?

↓ No

↓ Yes

No ↓

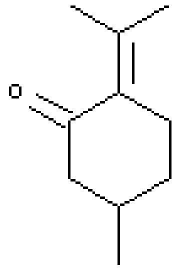
Additional data required

"No safety concern"

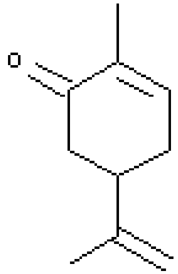
Expansion of the DT

- 1978-present –volume of toxicology and metabolism data ↑
  - increased data → more branches of DT
  - revise steps in the current DT
    - Step 18h (four or more carbons bonded to a ketone, DT II
    - Step 18h (only 2-hexanone with methyl substitution at C-3 or C-4 DT III or DT IV
  - eliminate steps no longer valid
    - Is the substances endogenous?
    - Terpene branch unnecessary

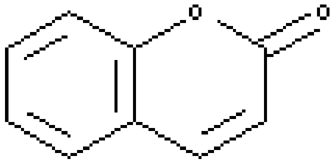
DT-Reconciling New Knowledge



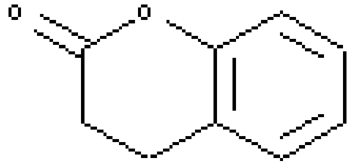
Pulegone
DT Class II
“enonal” intoxication
NOAEL 10 mg/kg/d



Carvone
DT Class II
ω-oxid. & reduction
detoxication
NOAEL 750 mg/kg/d

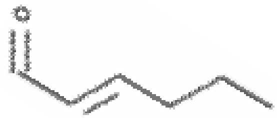


Coumarin
DT Class III
No hydrolysis
epoxidation-rat
intoxication
NOAEL <50 mg/kg/d

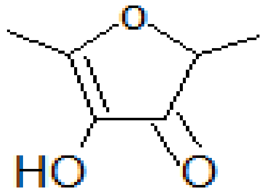


Dihydrocoumarin
DT Class III
hydrolysis to 2-phenylpropionic acid
detoxication
NOAEL 150 mg/kg/d

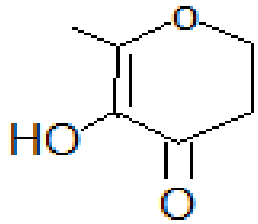
Citral
DT Class I
ω- & ald. oxid. To yield
diacid
Detoxication
NOAEL 200 mg/kg/d



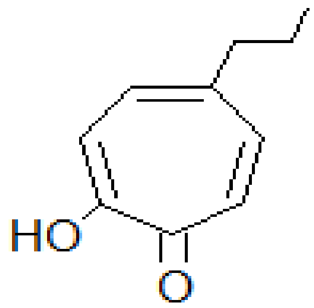
2-Hexenal
DT Class I
ald. oxid. & glutathione
conjugation
Detoxication
NOAEL 80 mg/kg/d



furaneol



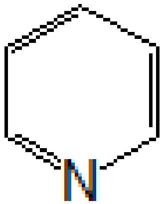
maltol

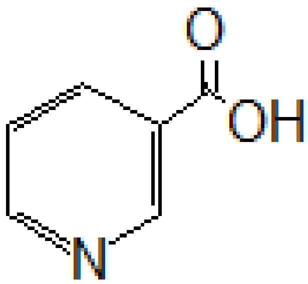


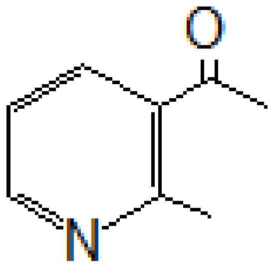
hinokitiol

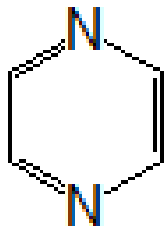
DT Class			
Genotoxicity (ex)	+ AMS, + in vivo MN (ip)	+AMS +/- In vivo MN(ip)	+AMS, +in vivo MN (ip)
Metabolism	Glu acid conj. (h)	Glu acid conj. (h)	Fe+++ complex/stored
Biochemistry	Fe+++ complex/SOD/OH.	Fe+++ complex/SOD/OH.	Fe+++ complex/SOD/OH.
Kow	<1.5	<1.5	3.0
2-yr Bioassay	Not Carcinogenic	Not Carcinogenic	Not Carcinogenic Splenic effects
NOAEL	150 mg/kg/d	200 mg/kg/d	<50 mg/kg/d
Proposed DT Class	II	II	III

Heteroaromatics









NOAEL mg/kg	5	250	150	40
Proposed Class	IV	II	II	III

Expanded/Revised DT Revisions

- Revise “trunk”- steps lacking biochemical basis eliminated
  - Biological normality Step 1
  - Common component of food Step 22

- Increase elements (Step 3) C, H, O, N, divalent S, higher oxid. S, Cl, F, and P in a biologically stable oxidation state

Expanded/Revised DT

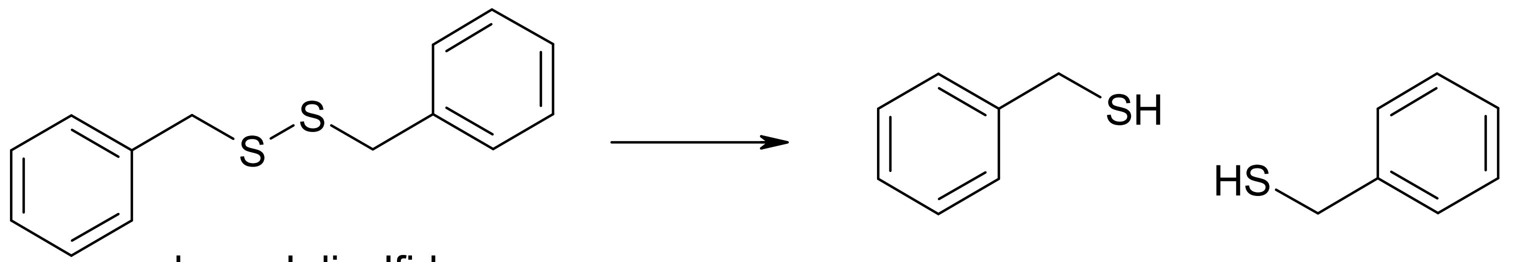
- Evaluate absorption first
  - Effect of functional group, size, etc.
    - Effect on other nutrient absorption (Fe, Ca)
- "Main branches" of DT
  - Branched-chain aliphatics and alicyclics treated in same branch
  - Aromatic and heteroaromatic-treated separately

Multiple Factors Used inExpanded DT

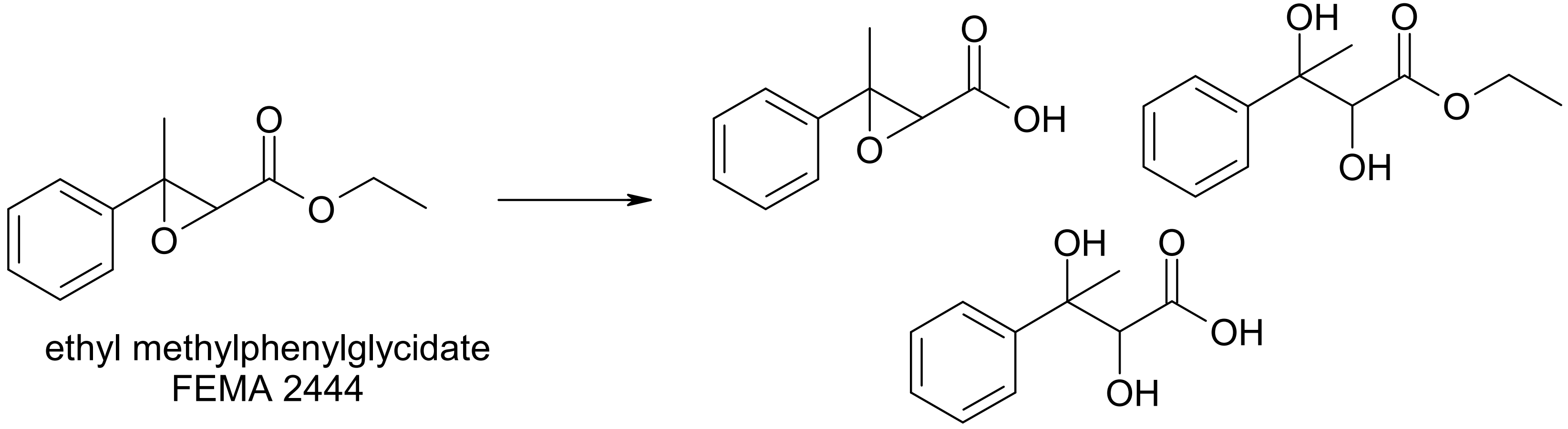
- Complex Integration Process
  - Functional group
  - Skeletal structure
  - Functional moiety (exocyclic isopropylidene vicinal to ketone)
  - Presence or absence of other functional groups
  - Extent of conjugation
  - Impact of electron donating groups
  - Positional & geometric isomers

Reactivity Prior to Metabolism

- Step 1 Does the substance contain functional groups that are predicted or known to react (e.g., hydrolyze, reduce or oxidize) under conditions present in the gastric & intestinal compartments or in circulation prior to first-pass metabolism?



benzyl disulfide
FEMA 3617



ethyl methylphenylglycidate
FEMA 2444

Anticipated TTC Changes

Old DT Class

Class	5th% NOEL, mg/kg/d	TTC, ug/p/d	% flavoring substances in class
I	3.0	1800	80%
II	0.91	544	8%
III	0.15	90	12%

Expanded DT

Class	Approx. 5% NOEL mg/kg/d	TTC, ug/p/d	NOEL Range mmol/kg/d MW=200
I	50	30000	>2.5
II	10	6000	0.8-2.5
III	1.5	900	0.25-0.75
IV	0.5	300	0.05-0.25
V	0.1	60	<0.05

Structural Class Changes ?

trans-Anethole III-II
Ethyl methylphenylglycidate III→I
Dihydrocoumarin III→I
4-Methyl-5-thiazoleethanol III→III
Isoeugenyl methyl ether III→I
Ethyl 3-phenylglycidate III→I
p-Propylanisole III→II
6-Methylcoumarin III→II
Methyl beta-naphthyl ketone ?
Methyl N-methylanthranilate III→I
4-Methylbiphenyl III→II
4,5,6,7-Tetrahydro-3,6-dimethylbenzofuran III
2-Ethyl-4,5-dimethyloxazole III→II?
Benzoin III→II
Bis(2-methyl-3 -furyl)tetrasulfide?
3-((2-Methyl-3-furyl)thio)-4-heptanone?
Estragole III
Tetrahydrofurfuryl alcohol III→II
Tetrahydrofurfuryl acetate III→II
Quinoline V

Thank You

Questions are welcome