

# Omsetning av Ethoxyquin in laks

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Bergen, 2-3 December 2008

# What is ethoxyquin?

- Heterocyclic aromatic organic compound
  - Derivative of quinoline
- **MW 217.34**
- Proton donor – antioxidant
  - **Numerous oxidation products**
- From 1950s
  - Herbicide/fungicide
  - Insecticide
  - Fertiliser
  - Anti-degradation agent (rubber production)
  - Food additive (chilli/paprika powders)
  - Preservative of dehydrated crops/animals feeds

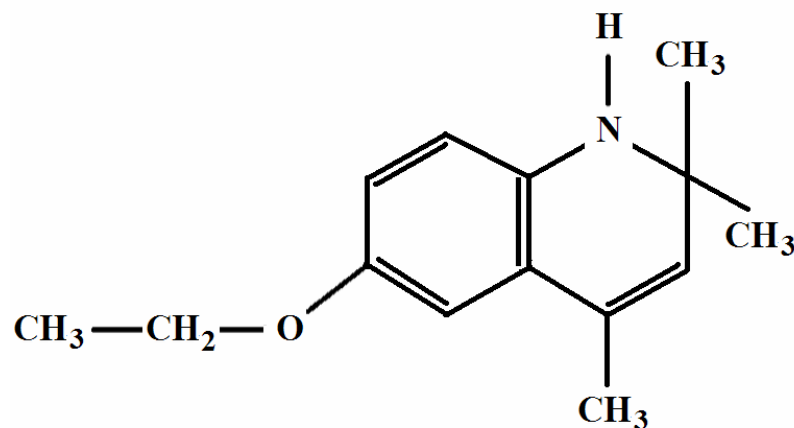
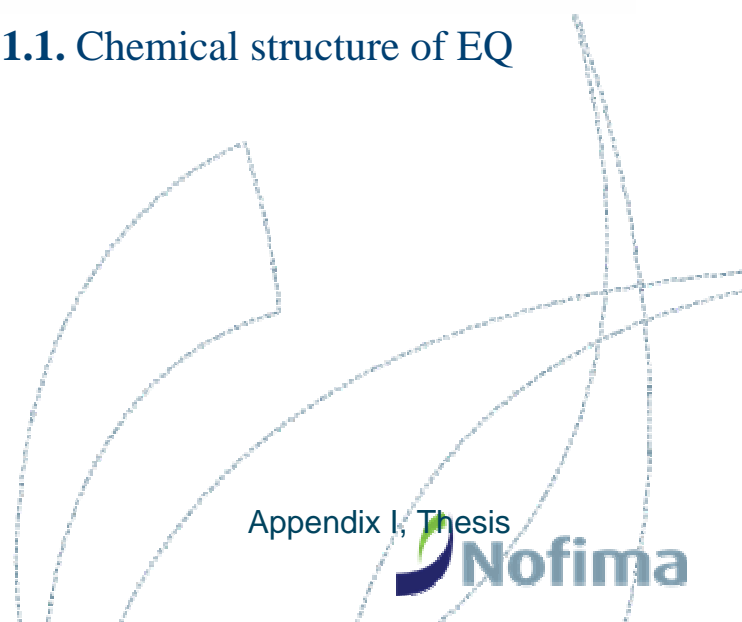


Figure 1.1. Chemical structure of EQ



Appendix I, Thesis

 Nofima

# Why add EQ to fishmeal?

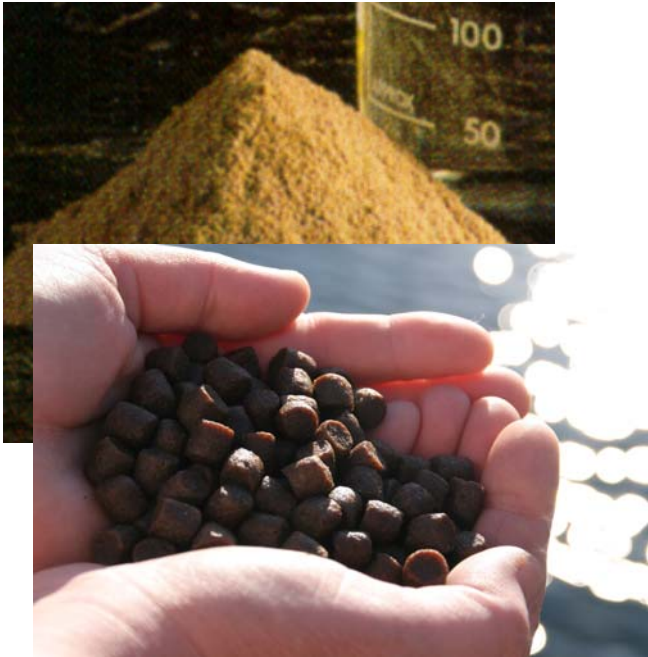


- IMO (International Maritime Organisation)
- Insurance companies

**Committee of Experts on  
the transport of  
dangerous goods (1999)  
100 mg EQ/kg fishmeal**

...February 11th 1966. The “South America” loaded with fishmeal was on its way from Peru to Poland when it caught fire. The heat made that some oil barrels ripped open which caused a real inferno. The ship was burning for days. The heat must have been enormous...

# Why add EQ to fishmeal?



- Fat-rich (unsaturated lipids)
- Auto-oxidation of lipids =
  - Formation of free radicals
  - Rancidity
  - Heating > Explosion under transport

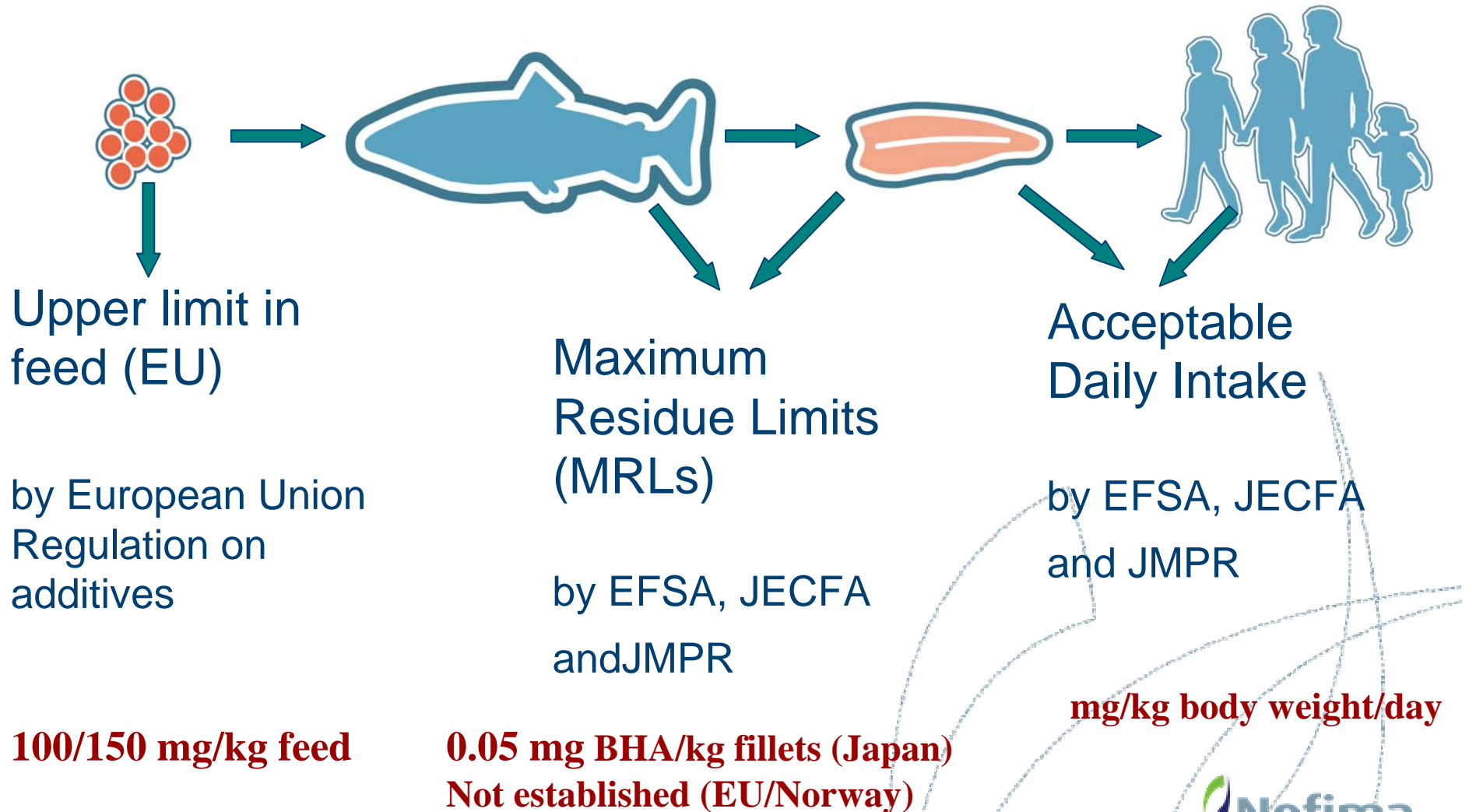
**Committee of Experts on the transport of dangerous goods (1999)**

**Upper limit of EQ, BHA, BHT  
European Union Regulation on additives (No 1831/2003)**

**100 mg EQ/kg fishmeal**

**150 mg EQ/kg feed**

# Legislation and food safety



# Acceptable Daily Intake (ADI)

is amount of compound consumed per kg body weight through food during each day of life span without causing any adverse effect on health

nomenclature	E-number	ADI, mg/kg body weight/day	Last JECFA evaluation
BHA	320	0-0.5	(1988)
BHT	321	0-0.3	(1995)
Ethoxyquin	324	0-0.005	(2005)
PG	310	0 – 1.4	(1996)
OG	311	0-0.5	(2000)

# Knowledge required for the legislation



How much is  
in fillets?



**Kinetics**  
**Metabolism**  
**Toxicity**



safe for fish?

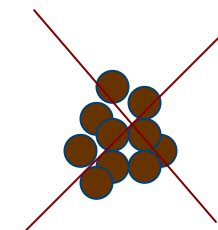
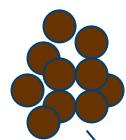


safe for  
consumers?



# Biological disposition of EQ in salmon: feeding trial

5 diets with 11, 18, 107, 1800 and 15000 mg EQ/kg feed  
15 tanks  
60 fish/tank  
5 fish per tank



reversed-phase HPLC → detection at 358/433 nm

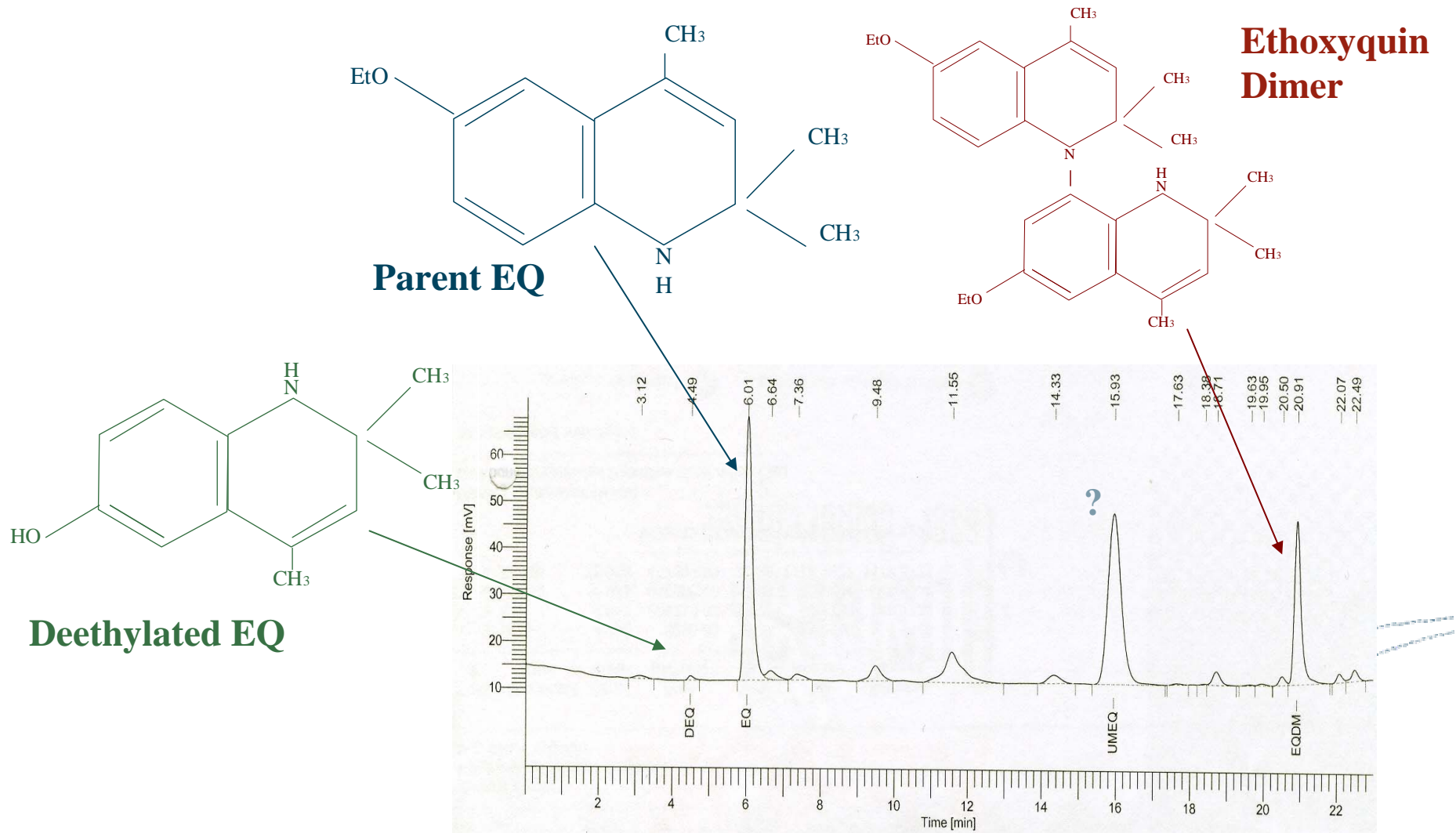
T, weeks

12

14



# Biological disposition of EQ in salmon: analytical tool (ISO/IEC 17025)



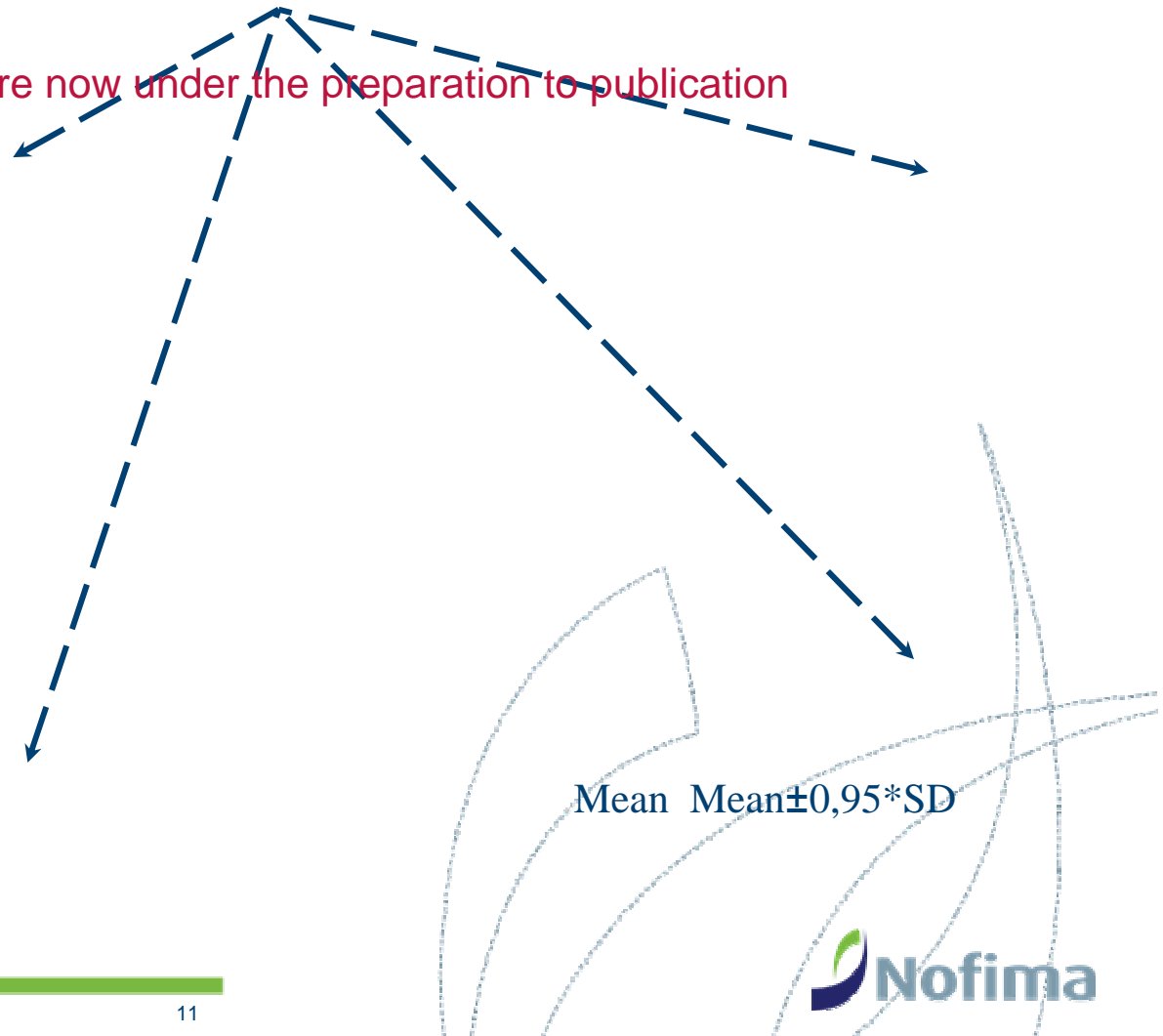
# Validation (Table 4.4.1.1)

	<b>EQ</b>	<b>EQDM</b>
Detection, nm	<b>358/433</b>	<b>358/433</b>
Limit of detection, µg/kg	<b>0.02</b>	<b>0.06</b>
Limit of quantification, µg/kg	<b>0.07</b>	<b>0.21</b>
Mean % recovery	<b>97</b>	<b>98</b>
Mean % precision	within-assay	<b>2</b>
	between-assays	<b>10</b>

# Biological disposition of EQ in salmon: tissue distribution of EQ

## Adipose tissue

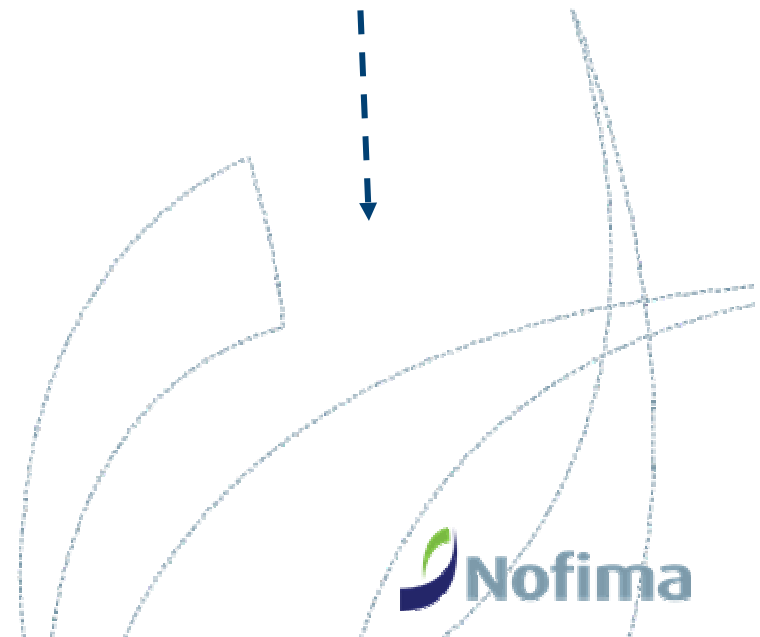
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# Biological disposition of EQ in salmon: tissue distribution of EQDM

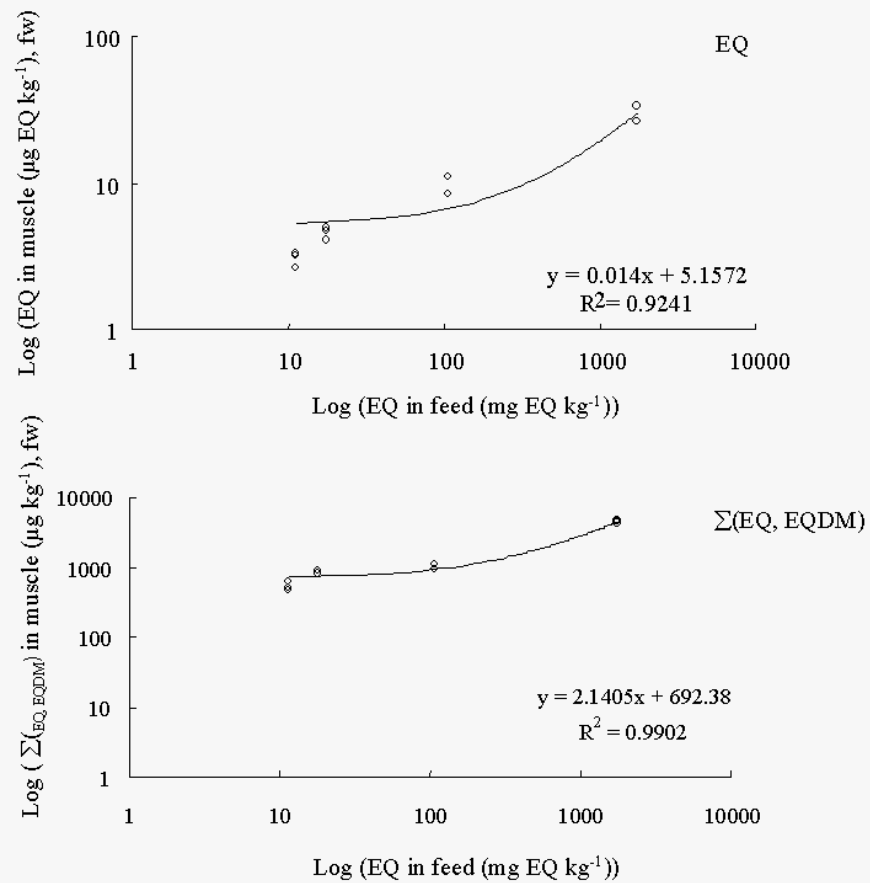
**Adipose**

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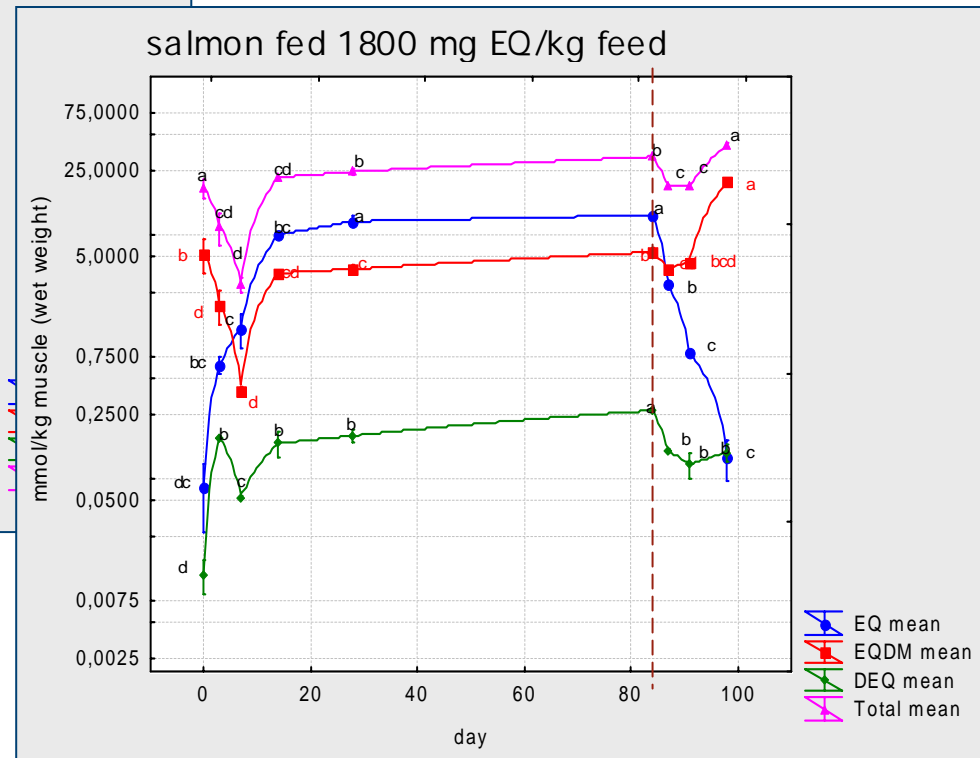
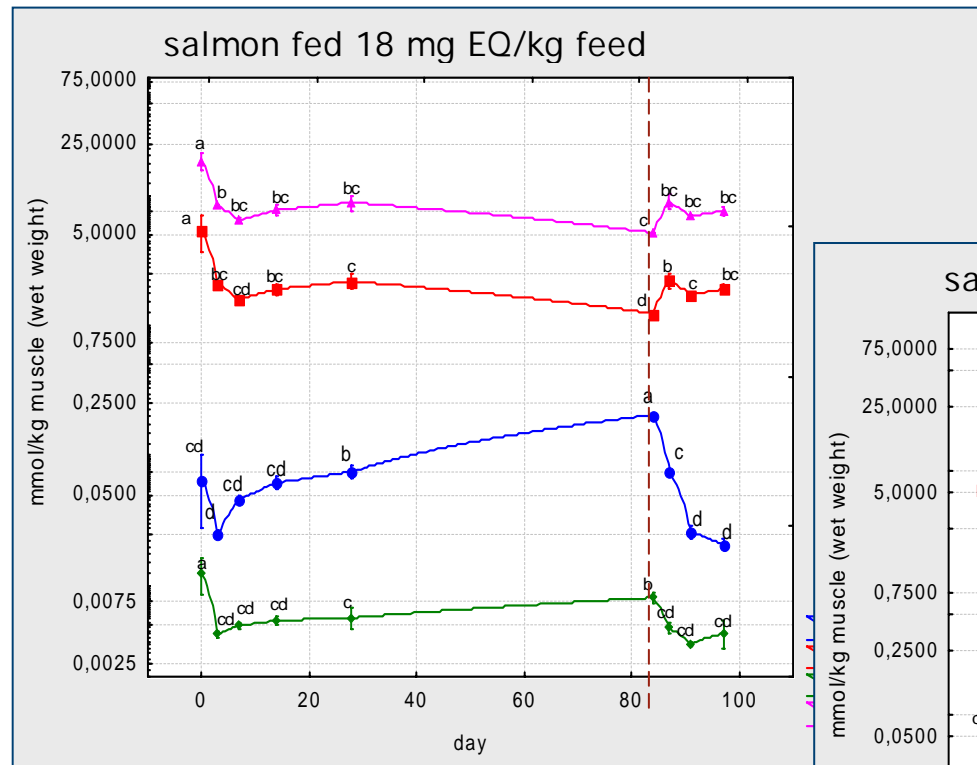


# Residue concentration ~ dietary level

FIG. 3. Dose-response curve of parent ethoxyquin (EQ) and of the sum of parent ethoxyquin and ethoxyquin dimer ( $\Sigma$  EQ, EQDM) retained in the muscle of Atlantic salmon after 2 week depuration.



# Results II. Kinetics in the muscle



# Kinetics of EQ and EQDM in muscle

FIG.1

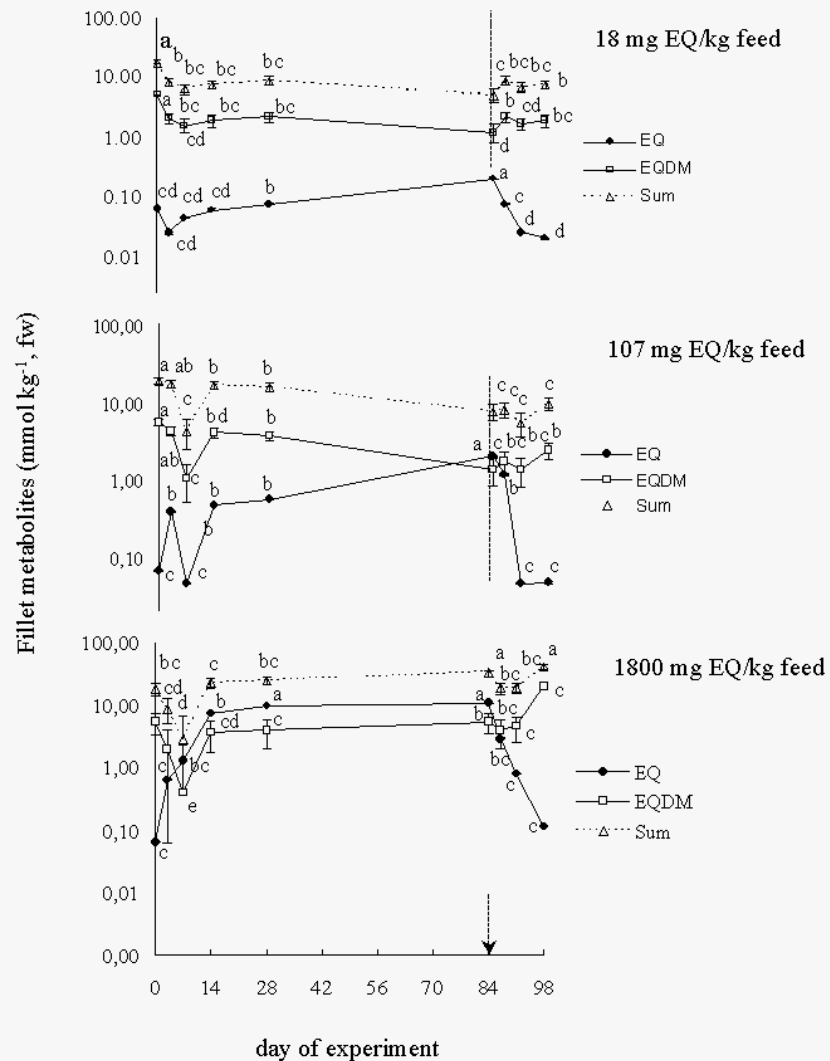


Table 4

- $t_{1/2} = 2.4$  days
- $k_{el} = 0.29$  day<sup>-1</sup>
- First-order kinetics
  - Rate ~conc
- $t_{1/2} = \infty? >2w$
- $k_{el} = 0$  day<sup>-1</sup>



# Biological disposition of EQ in salmon: comparison to other species

- No data from other fish species, except for silverhead (He and Ackman, 2000)
- EQDM was not identified earlier by Skaare and Roald (1977)
- Speculation about EQDM in rat and mice tissues (Burka et al., 1996; Sanders et al., 1996)
- Concentration of EQ in rat and mice muscle – low (Sanders et al., 1996)



# Biological disposition of EQ in salmon: comparison to other synth. antioxidants

- Synthetic antioxidants transfer from feeds into the fish
- BHT is not metabolised (Hamre and Bohne in *Final rapport for SYNTOX-project 143314*. Hamre 2006):
  - 8-10% of accumulated BHT excreted during depuration
  - Metabolism of BHT in all species, including a man – slow, 43 metabolites
- BHA is not accumulates in samon and almost undetectable after depuration period (Petri et al., 2007)
  - Metabolites of BHA were not investigated

# Theoretical Exposure to EQ from fish consumption

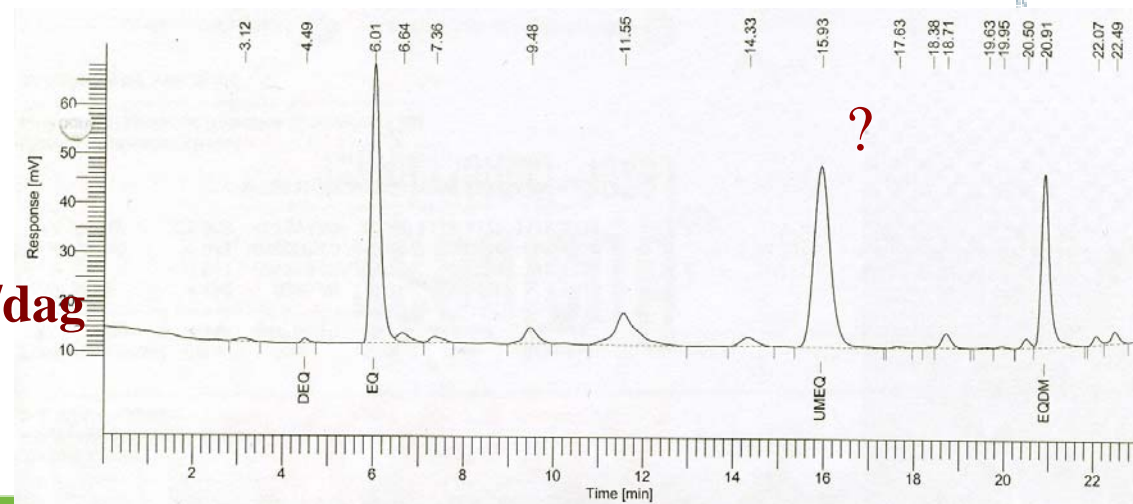
*BW (body weight)*      60 kg  
*C<sub>muscle</sub>*                estimated from dose-response curve  
*m (portion salmon)*    0.2 kg  
*f (number of meals)*    7 dager i uken  
 ADI for EQ                5000 ng/kg bw/day



$$\text{AverageDI} = \frac{C_{\text{muscle}} \times m \times f}{7 \text{ BW}}$$

**24 ng EQ/kg bw/dag**

**3390 ng EQ + EQDM /kg bw/dag**



# Ethoxyquin: Nofima is knowledgeable and experienced

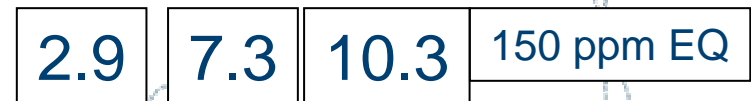
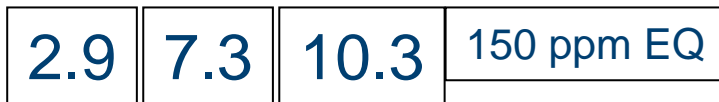


# Nofima: we know how – you know were

- Together for intelligent way of using EQ and its substitution where it possible
  - Mapping farmed species which are not accumulate EQ or its metabolites;
  - Determinate the feed components, which may increase disposition of EQ and decrease its accumulation;
  - Study detoxification mechanisms of EQ;
  - Exploire sustainable substitutions of EQ

# Nofima: A novel type of natural antioxidant is comparable to EQ in **krill meals**

Sorry, data is not published yet.



# List of publications

Bohne, V.J. Berdikova, Lundebye, A-K., Hamre, K. (2008). **Kinetics of the synthetic antioxidant EQ in the muscle of Atlantic salmon (*Salmo salar*, L).** *Food and Chemical Toxicology*, **46** (5), p.1834-1843.

Bohne, V.J. Berdikova, Hove, H., Hamre, K. (2007).  
**Simultaneous quantitative determination of the synthetic antioxidant ethoxyquin and its major metabolite in Atlantic salmon (*Salmo salar*, L), ethoxyquin dimer, by reversed-phase high-performance chromatography with fluorescence detection.** *AOAC International*, **90**(2), 587-597.

Bohne, V.J. Berdikova, Hamre, K., Arukwe, A. (2007). **Hepatic metabolism, phase I and II biotransformation enzymes in Atlantic salmon (*Salmo salar*, L) during a 12 week feeding period with graded levels of the synthetic antioxidant, ethoxyquin.** *Food and Chemical Toxicology*, **45** (5), 733-746.

Bohne, V.J. Berdikova, Hamre, K., Arukwe, A. (2006). **Hepatic biotransformation and metabolite profile during a 2-week depuration period in Atlantic Salmon fed graded levels of the synthetic antioxidant, ethoxyquin.**  
*Toxicological Sciences*, **93**(1), 11-21.