Omsetning av Ethoxyquin in laks

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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

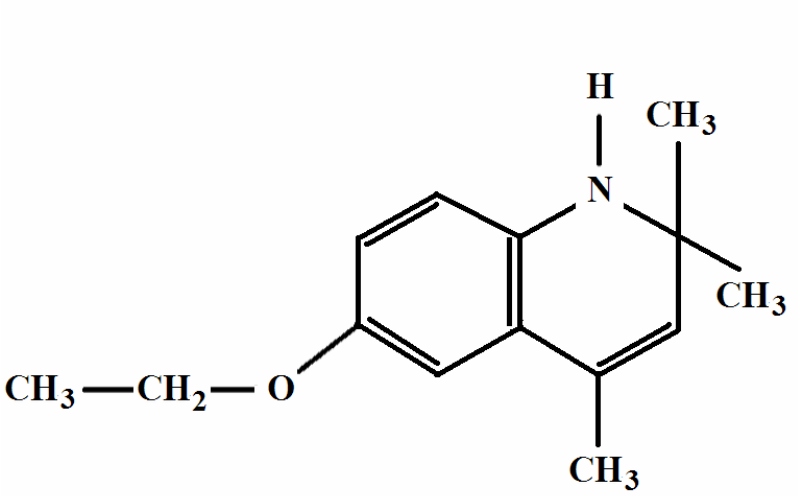
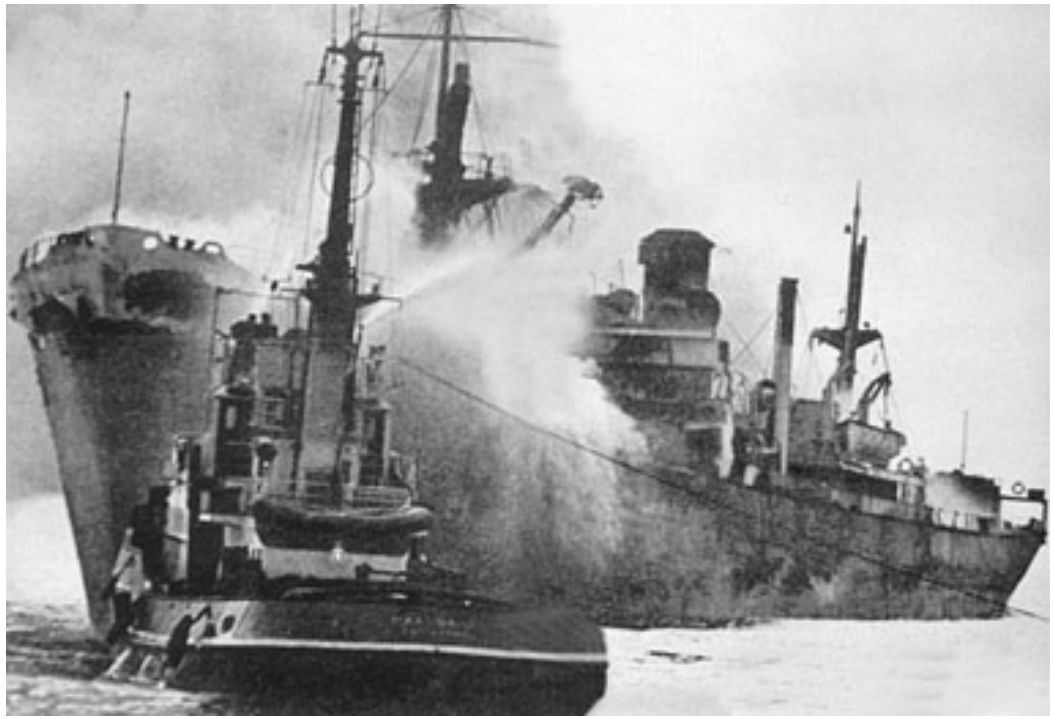


Figure 1.1. Chemical structure of EQ

Appendix I, Thesis

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

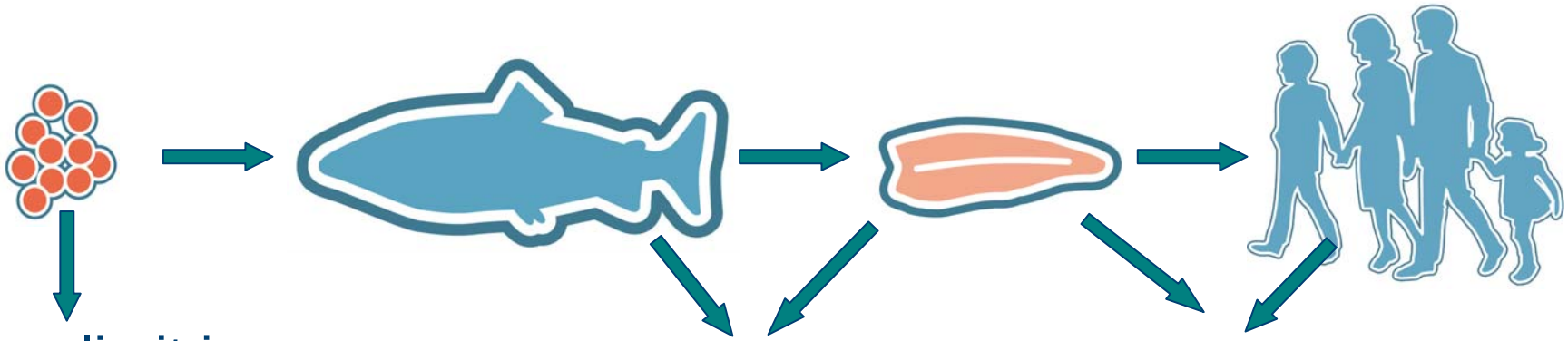
Committee of Experts on the transport of dangerous goods (1999)

100 mg EQ/kg fishmeal

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

150 mg EQ/kg feed

Legislation and food safety



Upper limit in feed (EU)

by European Union Regulation on additives

100/150 mg/kg feed

Maximum Residue Limits (MRLs)

by EFSA, JECFA andJMPR

0.05 mg BHA/kg fillets (Japan)
Not established (EU/Norway)

Acceptable Daily Intake

by EFSA, JECFA and JMPR

mg/kg body weight/day

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?

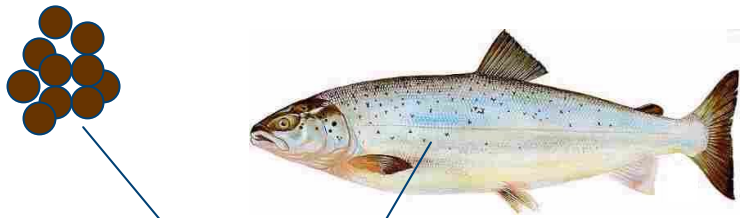
Kinetics
Metabolism
Toxicity

safe for fish?

safe for consumers?

Biological disposition of EQ in salmon: **feeding trial**

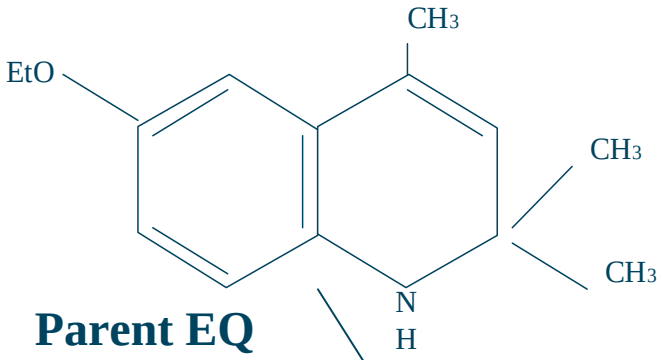
5 diets with **11, 18, 107, 1800 and 15000 mg EQ/kg feed**
15 tanks
60 fish/tank
5 fish per tank



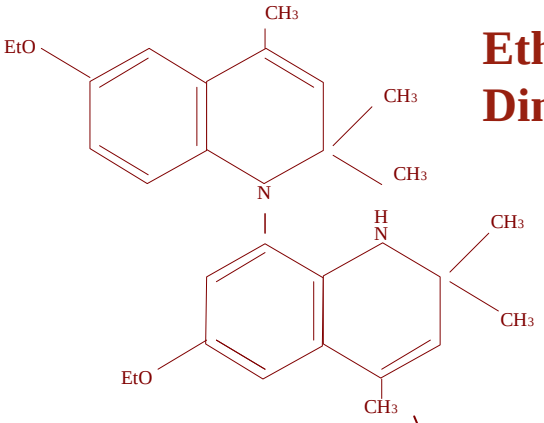
reversed-phase HPLC → detection at 358/433 nm

T, weeks 12 14

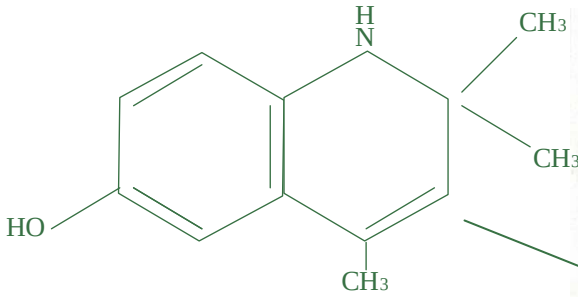
Biological disposition of EQ in salmon: **analytical tool (ISO/IEC 17025)**



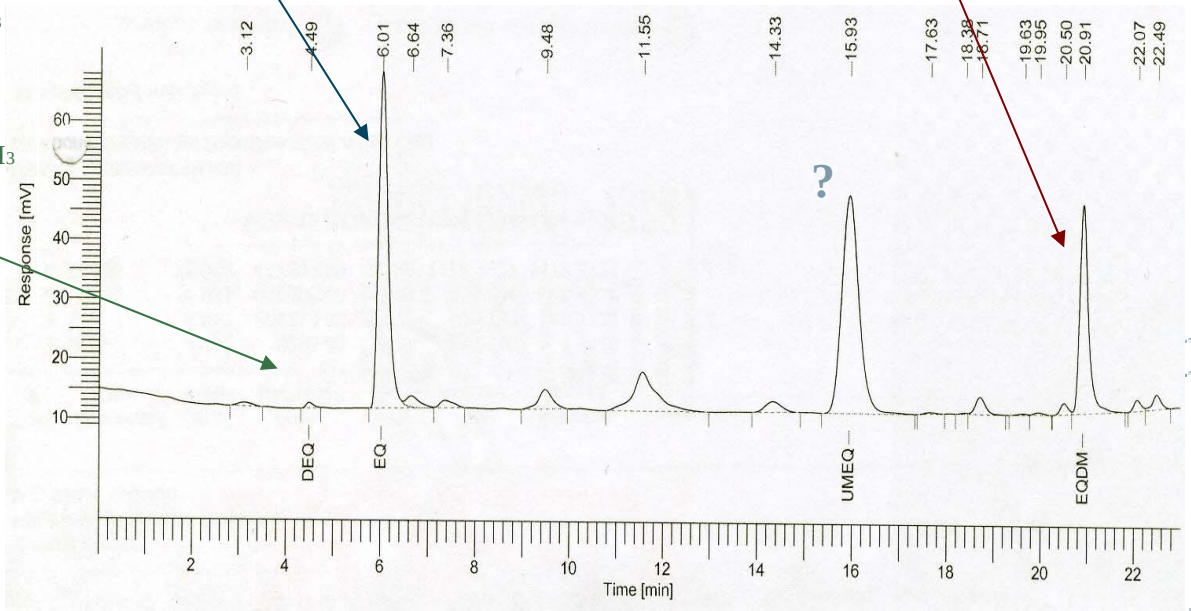
Parent EQ



Ethoxyquin Dimer



Deethylated EQ



Validation (Table 4.4.1.1)

		EQ	EQDM
Detection, nm		358/433	358/433
Limit of detection, µg/kg		0.02	0.06
Limit of quantification, µg/kg		0.07	0.21
Mean % recovery		97	98
Mean % precision	within-assay	11	2
	between-assays	21	10

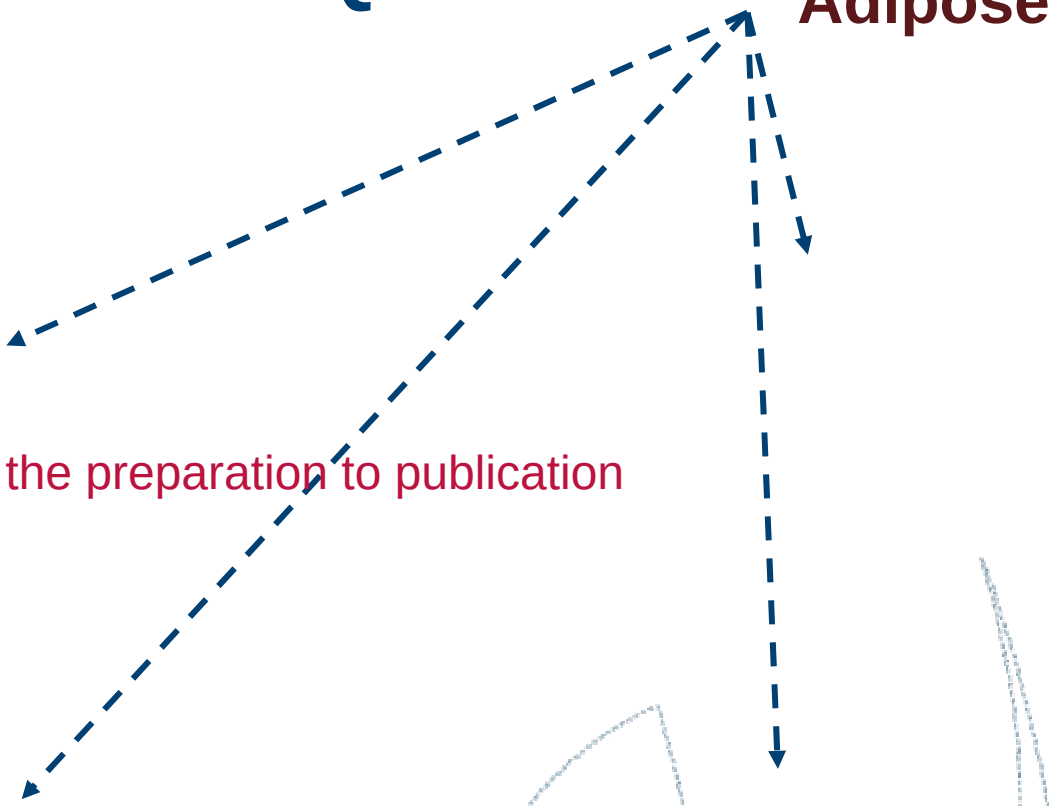
Biological disposition of EQ in salmon: **tissue distribution of EQ**

Adipose tissue

Sorry, the data are now under the preparation to publication

Mean Mean±0,95*SD

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



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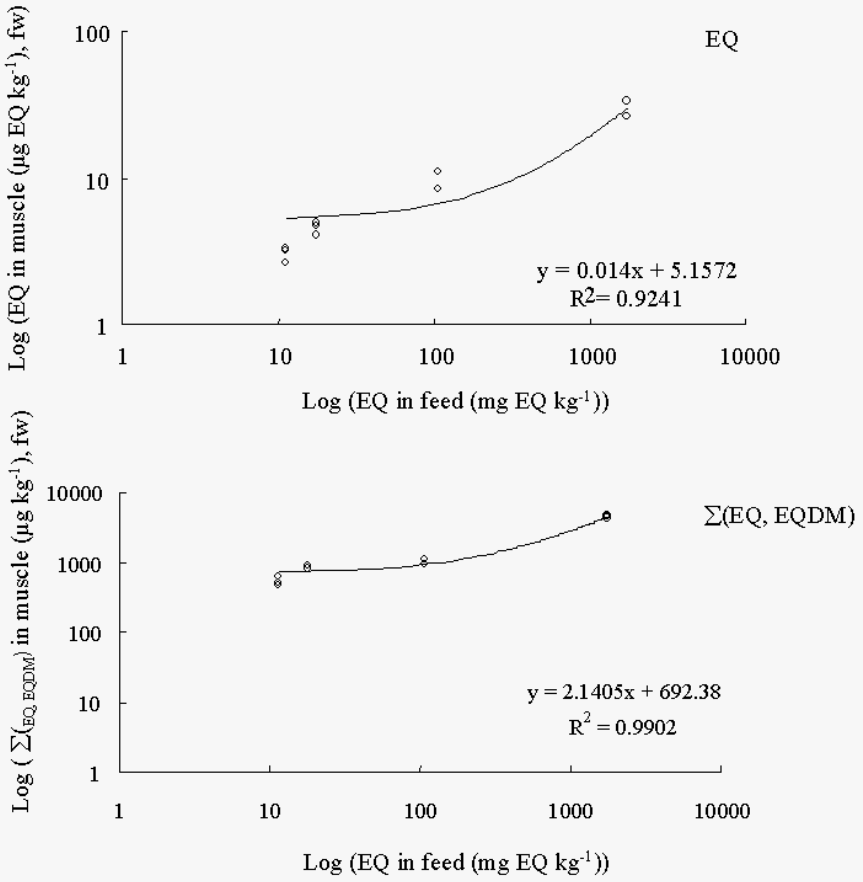
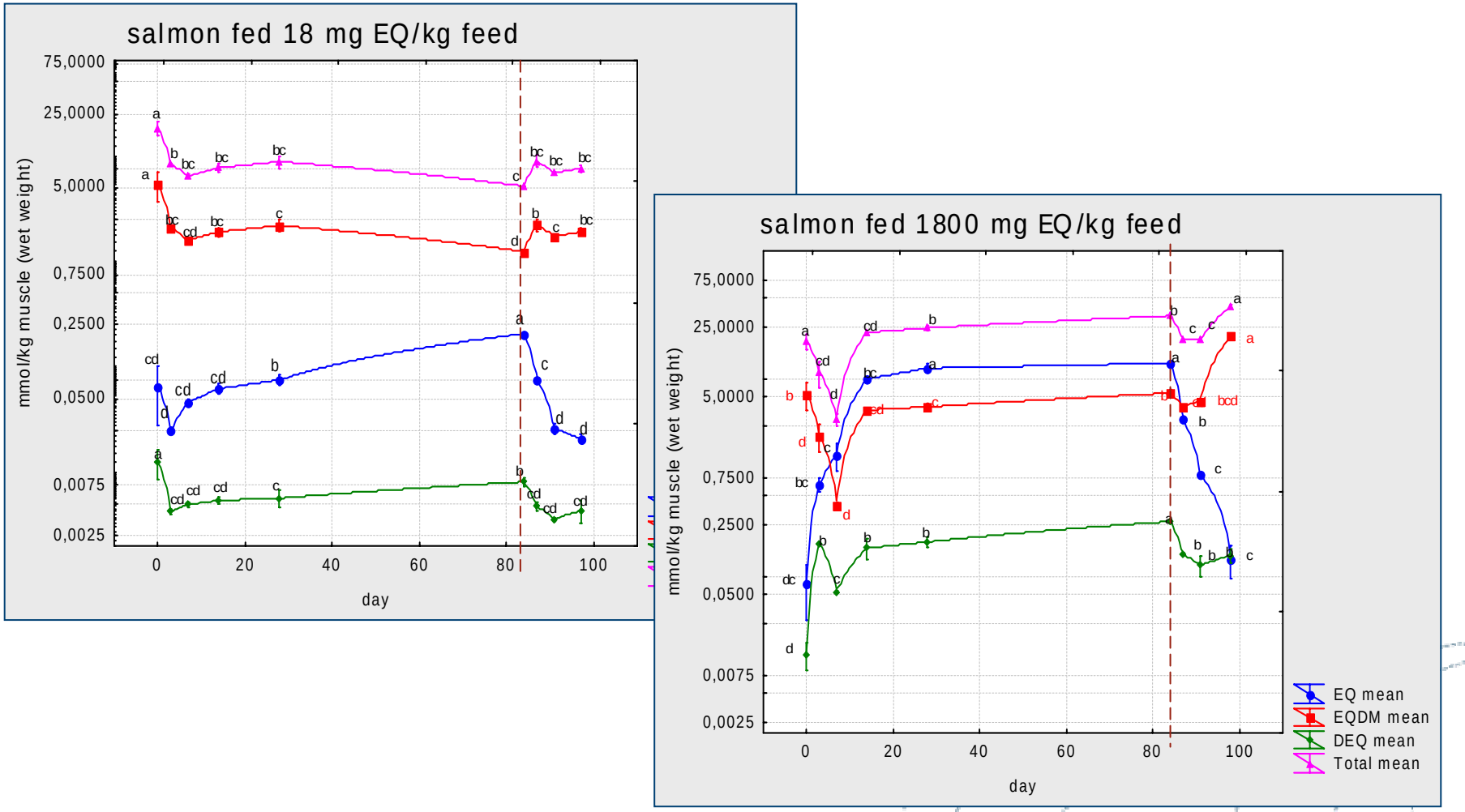
Residue concentration ~ dietary level

FIG. 3. Dose-response curve of parent ethoxyquin (EQ) and of the sum of parent ethoxyquin and ethoxyquin dimer (∑ 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

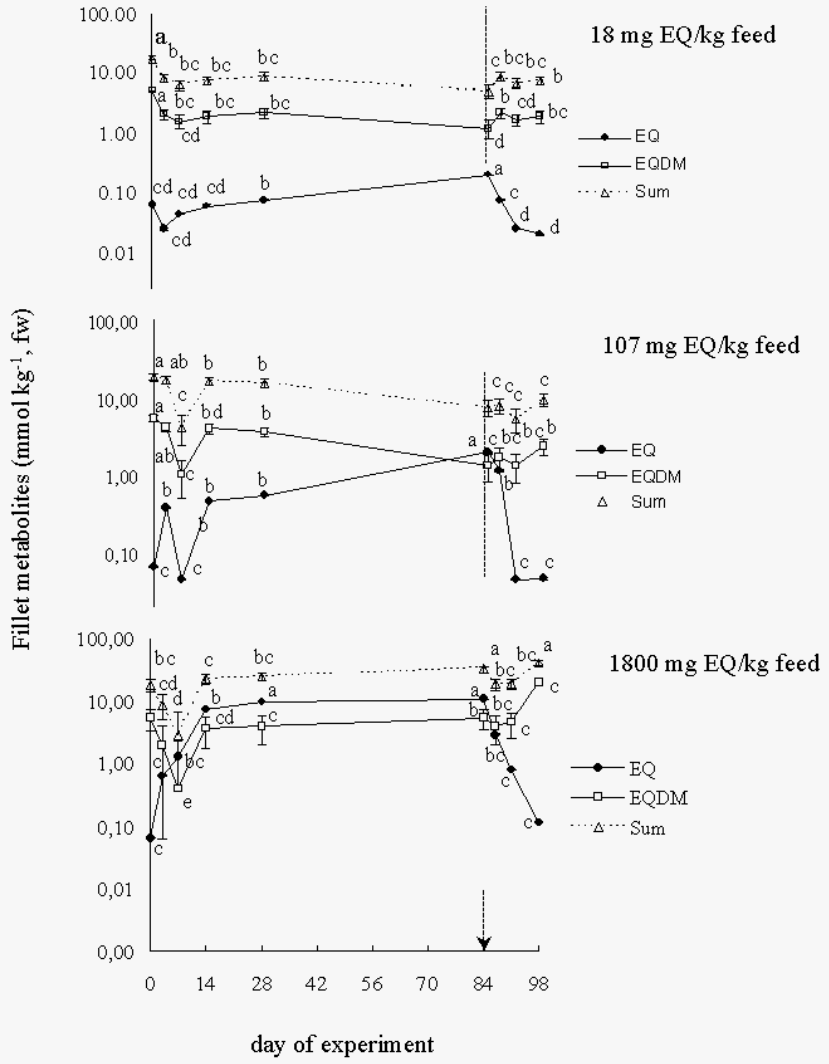


Table 4

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

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

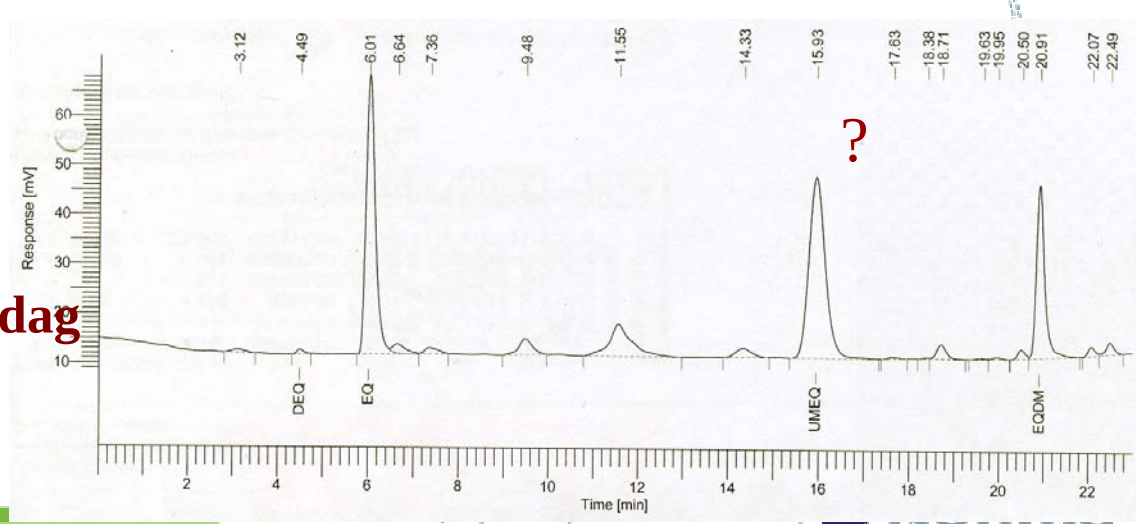
Theoretical Exposure to EQ from fish consumption

BW (body weight)	60 kg
C_{muscle}	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

$$AverageDI = \frac{C_{muscle} \; x \; m \; x \; f}{7 \; BW}$$

24 ng EQ/kg bw/dag

3390 ng EQ + EQDM /kg bw/dag



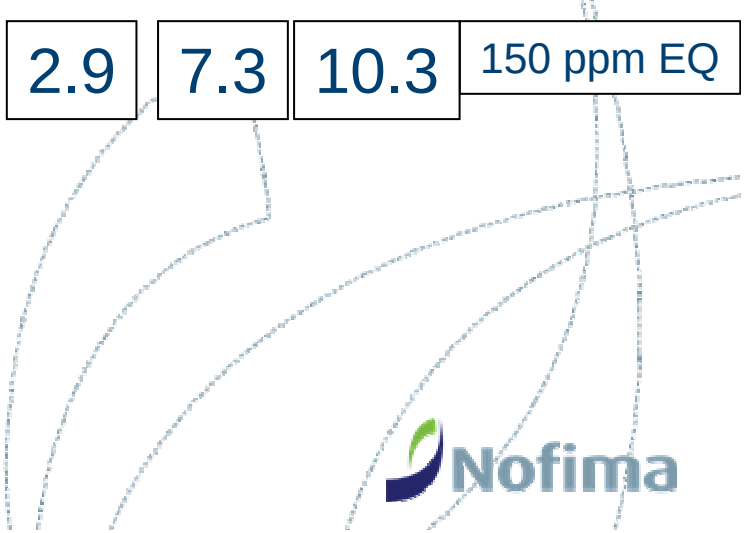
Ethoxyquin: Nofima is knowledgeble and experienced

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 detoxyfication 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.