Species Diversity of AHR-CYP1A Signaling Pathways: Toward Risk Assessment of Dioxin-like Compounds in Wildlife

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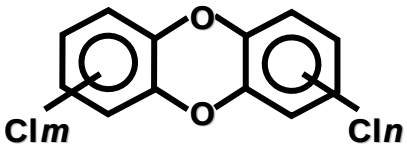
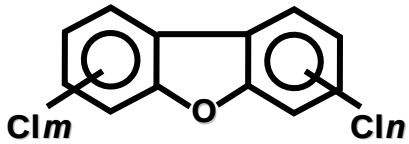
Dioxins and related compounds



m + *n* = 1 - 8

PCDDs

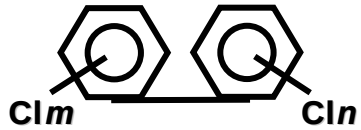
(Polychlorinated dibenzo-*p*-dioxins)



m + *n* = 1 - 8

PCDFs

(Polychlorinated dibenzofurans)



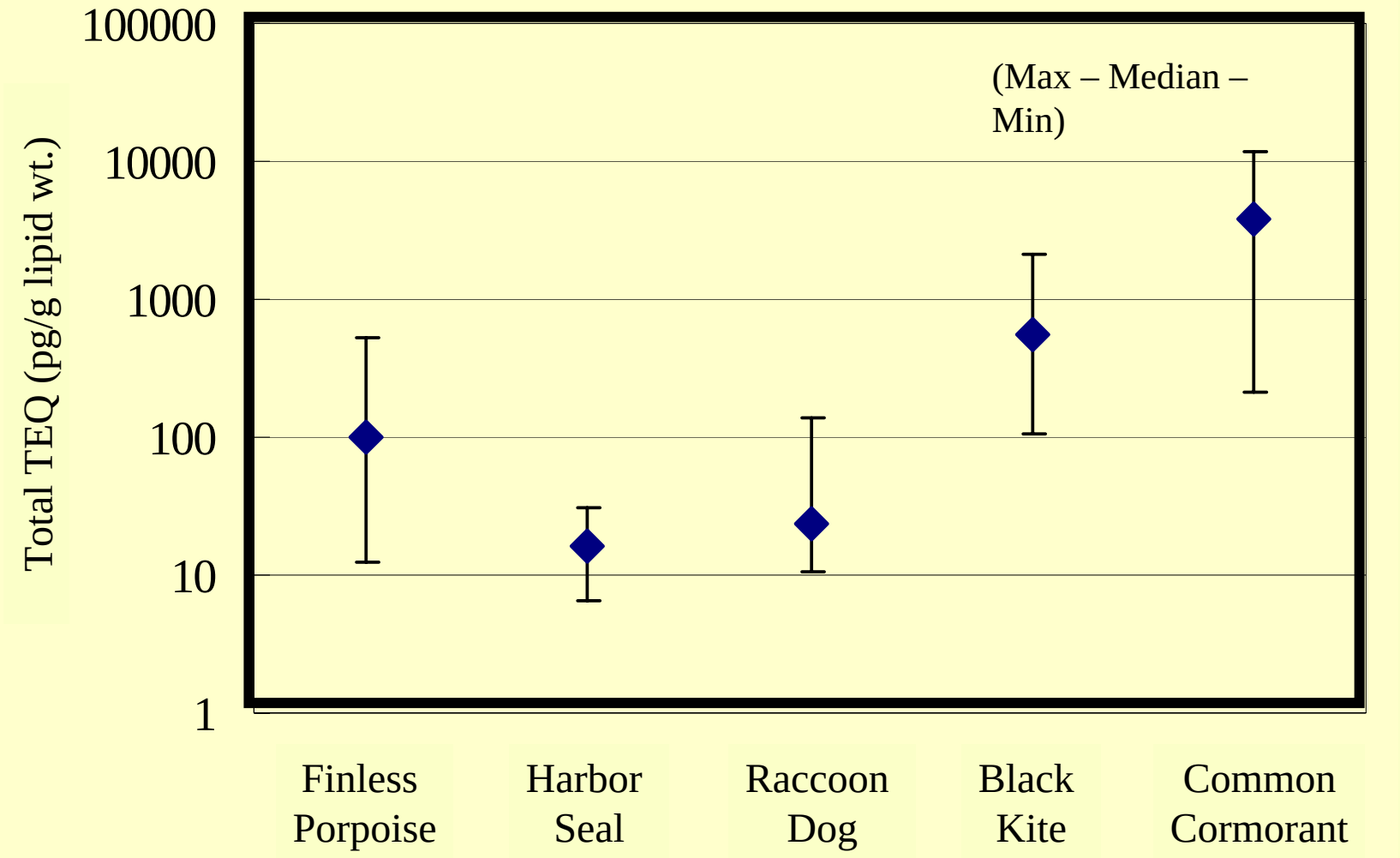
m + *n* = 1 - 10

Coplanar PCBs

(Coplanar polychlorinated biphenyls)

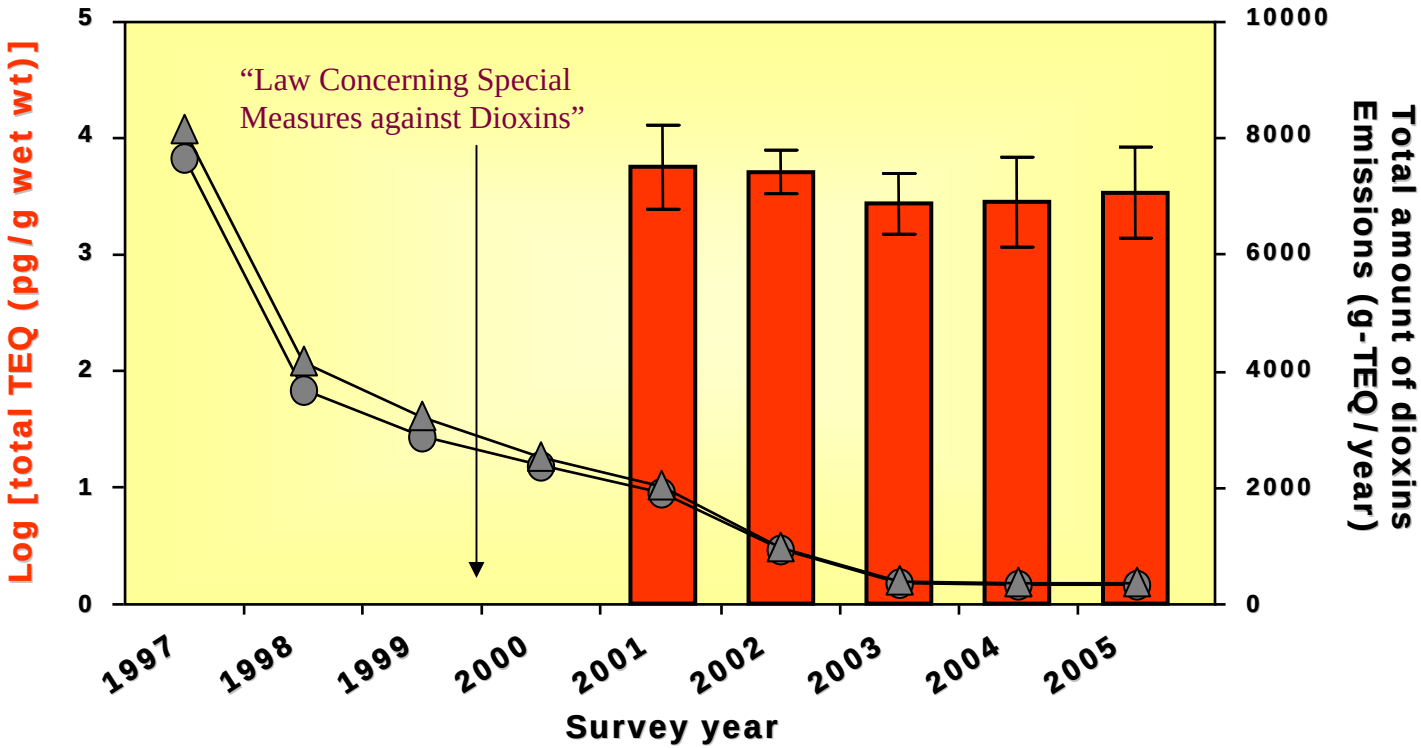
- **widely distributed in the environment**
- **persistent and lipophilic**
- **bioaccumulative**
- **highly toxic**

TEQ in adipose tissue of wildlife from Japan



Data from “Survey on the State of Dioxin Accumulation in Wildlife” (MOE)

Temporal trends of total TEQ in the liver of common cormorants from Lake Biwa and estimates of dioxins emission in Japan



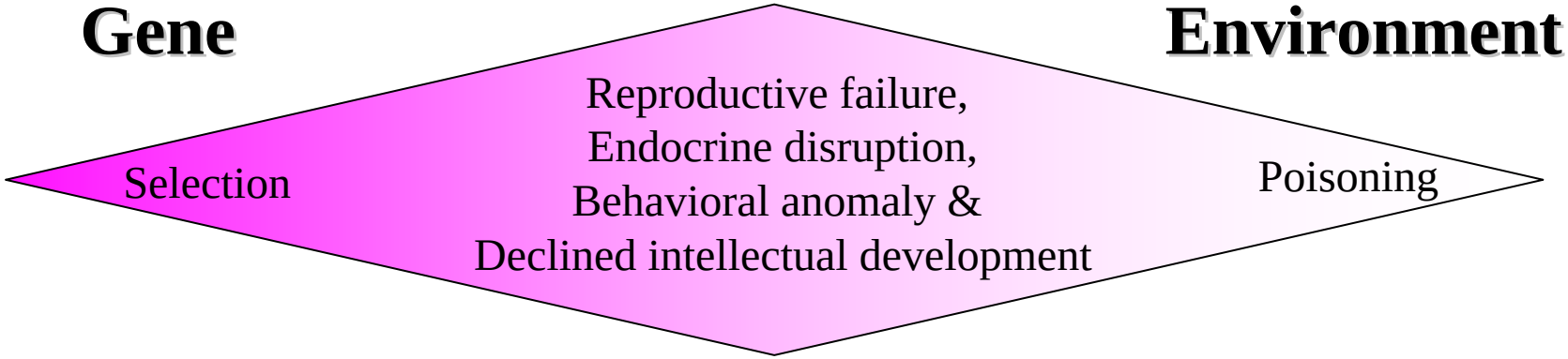
Bars show total TEQ (mean±SD) in the liver of cormorants.
Dioxins emission is represented as line plots of minimum (circle) and maximum (triangle).

An estimate from the national survey on dioxins emission inventory: 1900-2000 g-TEQ/y in 2001 to 320-350 g-TEQ/y in 2005.

TEQ levels did not decrease in wildlife during the corresponding period.

Data from "Survey on the State of Dioxin Accumulation in Wildlife" (MOE)

Two factors influencing health of organisms



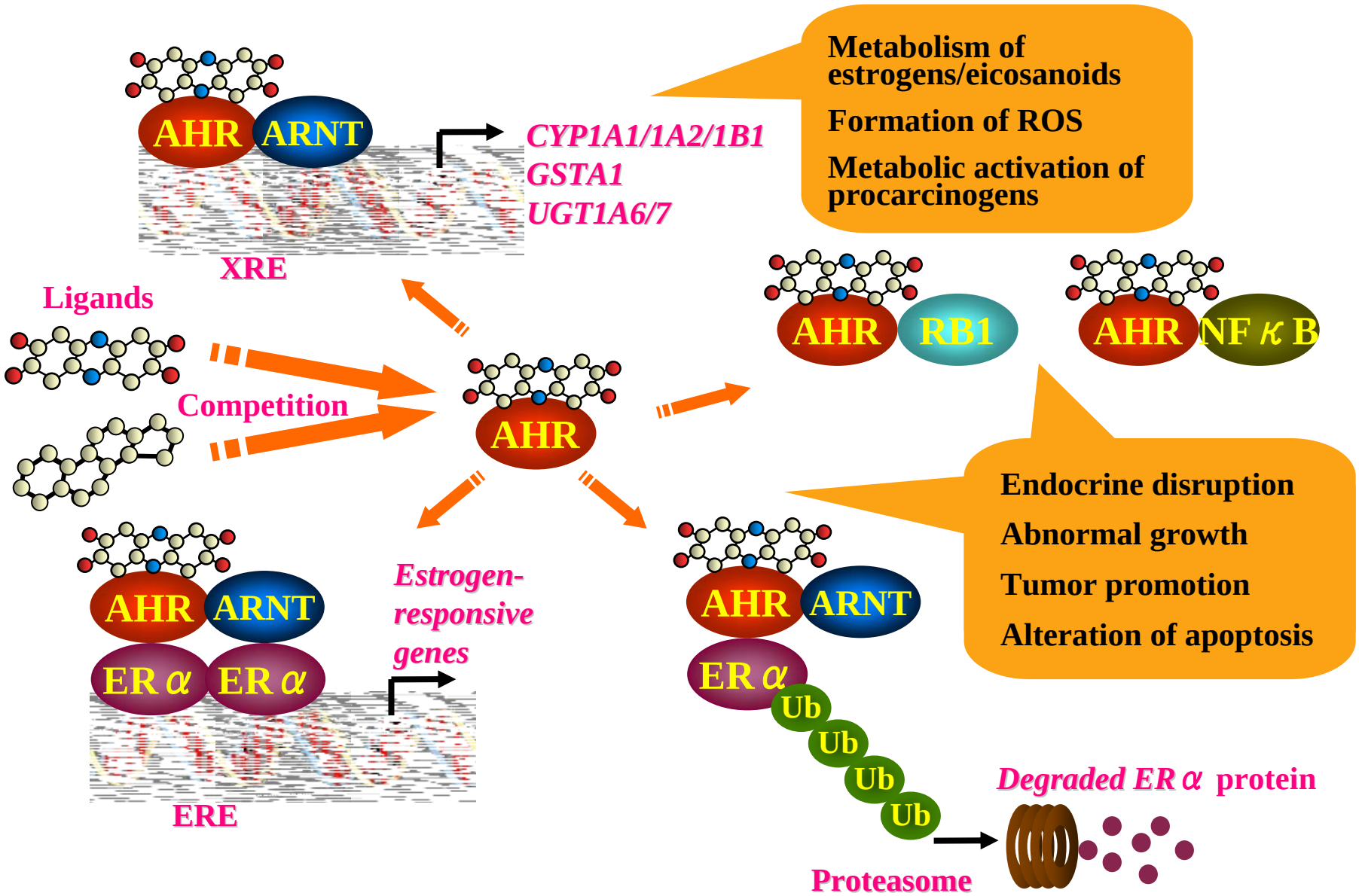
Risk $= F$ [**Environmental Factor Exposure High/Low**] [**Genetic Factor Sensitivity High/Low**]

Interspecies differences in sensitivity of toxicological responses to chemicals

e.g. **Guinea pig is more (5000-fold) sensitive to dioxins than hamster.**

Understanding genetic factor is necessary to assess the risk of chemicals in wildlife.

CYP1 induction & other effects mediated by dioxin-activated AHR



(Ohtake *et al*., 2003; Nebert and Dalton, 2006; Ohtake *et al*., 2007)

Is the AHR a good "biomarker of sensitivity" ?

- **Dioxins cause toxicity through activation of the AHR-dependent signal pathway (Poland *et al*., 1976).**
- **AHR knock-out mice are much less sensitive to the biochemical, lethal, and teratogenic effects of TCDD than wild-type mice (Fernandez-Salguero *et al*., 1996; Mimura *et al*., 1997; Peters *et al*., 1999).**
- **The 5- to 15-fold difference in TCDD sensitivity of two mouse strains can be explained by the difference in TCDD-binding affinities of their respective AHR proteins (Okey *et al*., 1989; Poland *et al*., 1994).**

AHR can be a critical determinant for the sensitivity to dioxin and the mechanism of dioxin action.

Much less information is available for the function of AHR in species other than limited model animals.

Comparative study on the AHR function may provide more information on the molecular mechanisms of dioxin-sensitivity.

Objectives

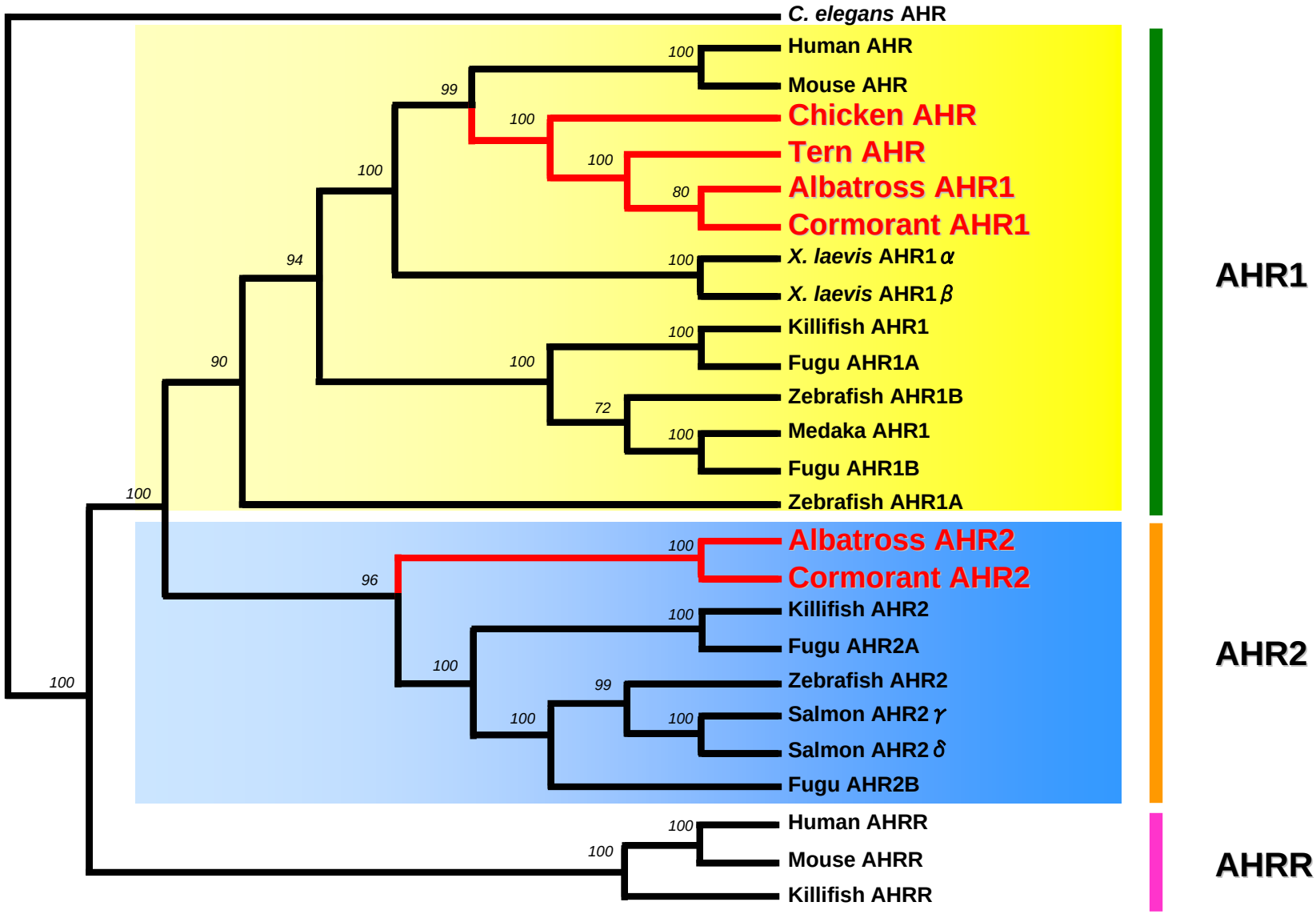
1) **To characterize the function of AHRs of avian and aquatic mammalian species,**

   the present study measured the species-specific responses of AHR - CYP1A signaling induced by dioxins through an *in vitro* reporter gene assay system.

2) **To evaluate the validity of the constructed in vitro reporter gene assay system,**

   relationships between TEQ and CYP1A expression levels were examined in the wild population of respective species, and compared with those of the *in vitro* dose-responses of dioxins.

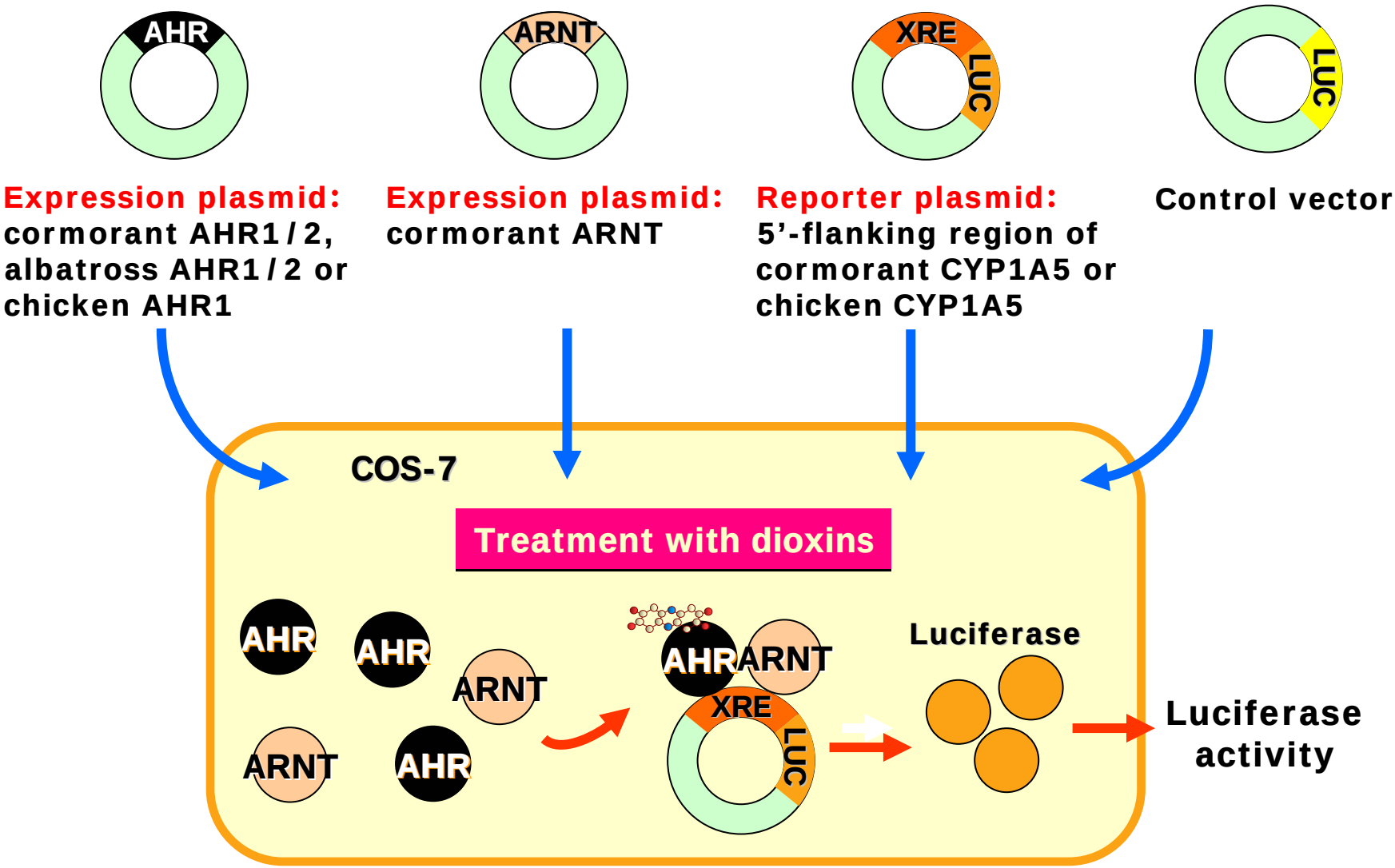
Phylogenetic analysis of AHRs from vertebrate species



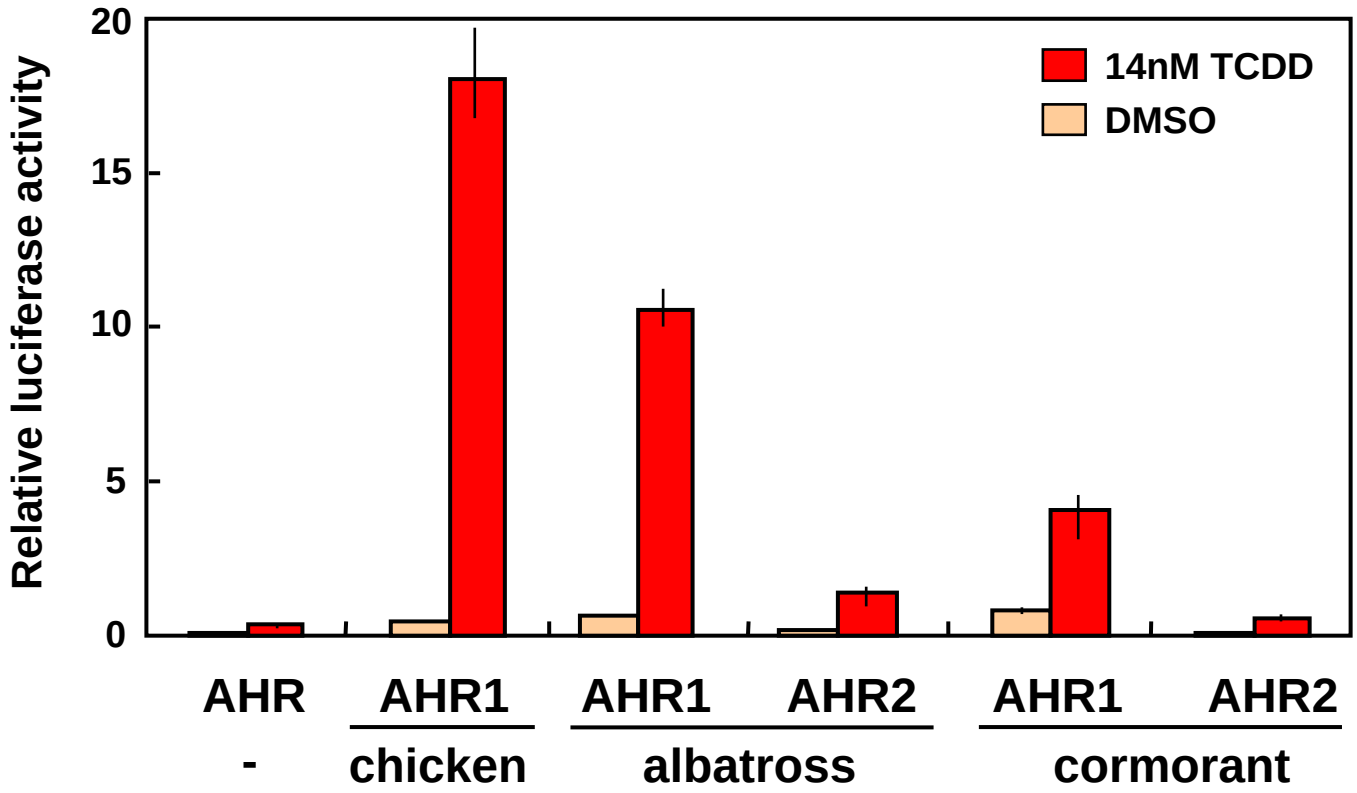
AHR2 was identified in vertebrate species other than fish.

Avian AHR2s exhibited only approximately 30 % identities with AHR1s and fish AHR2s.

Transactivation analysis of AHR: *in vitro* reporter gene assay



Comparison of transcriptional activities among AHR isoforms

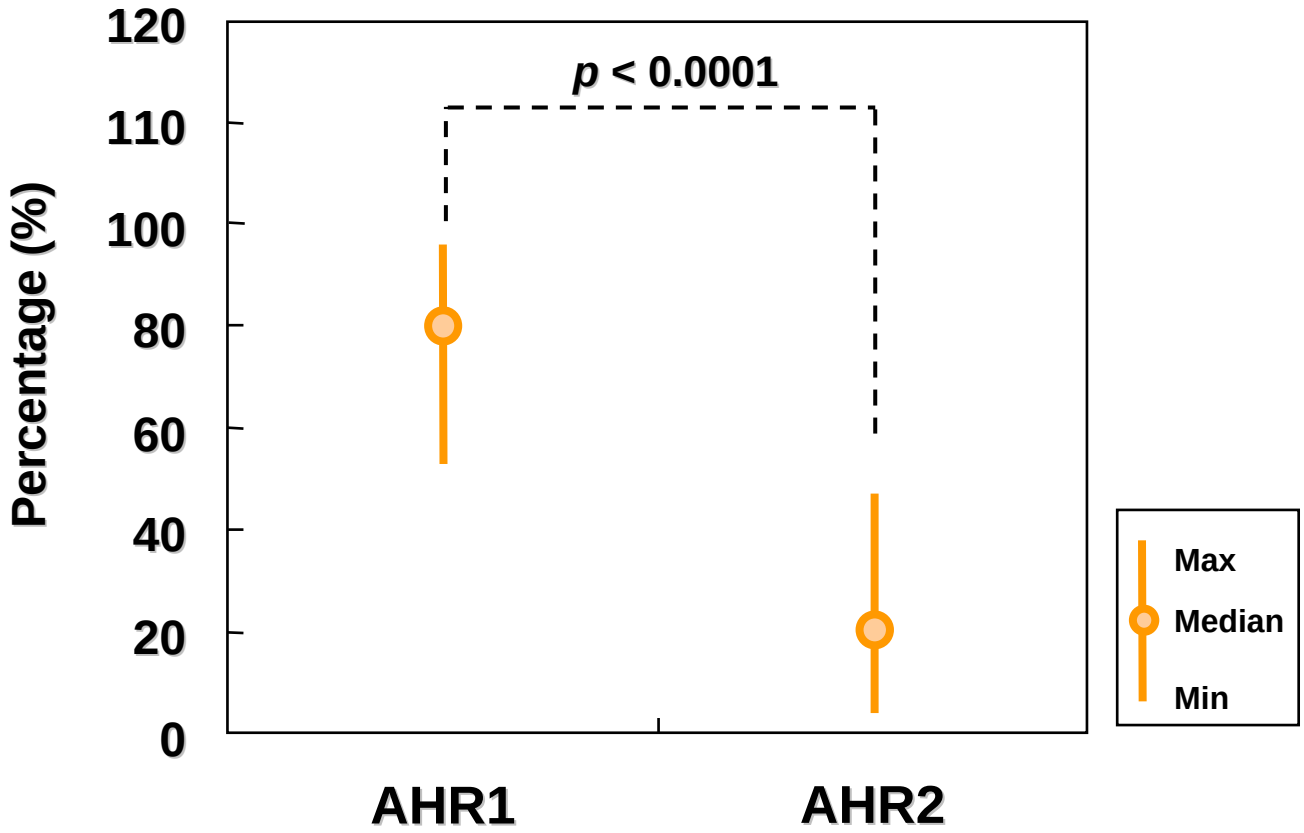


AHR1: Chicken AHR1 > Albatross AHR1 > Cormorant AHR1

AHR2: Low transactivation potency

AHR2 may be less functional from the point of view of CYP1A5 induction by dioxin.

Comparison of hepatic mRNA expression levels between AHR1 and AHR2 in the wild cormorant population from Lake Biwa



AHR1 is the dominant form of avian AHR.

Seal AHR

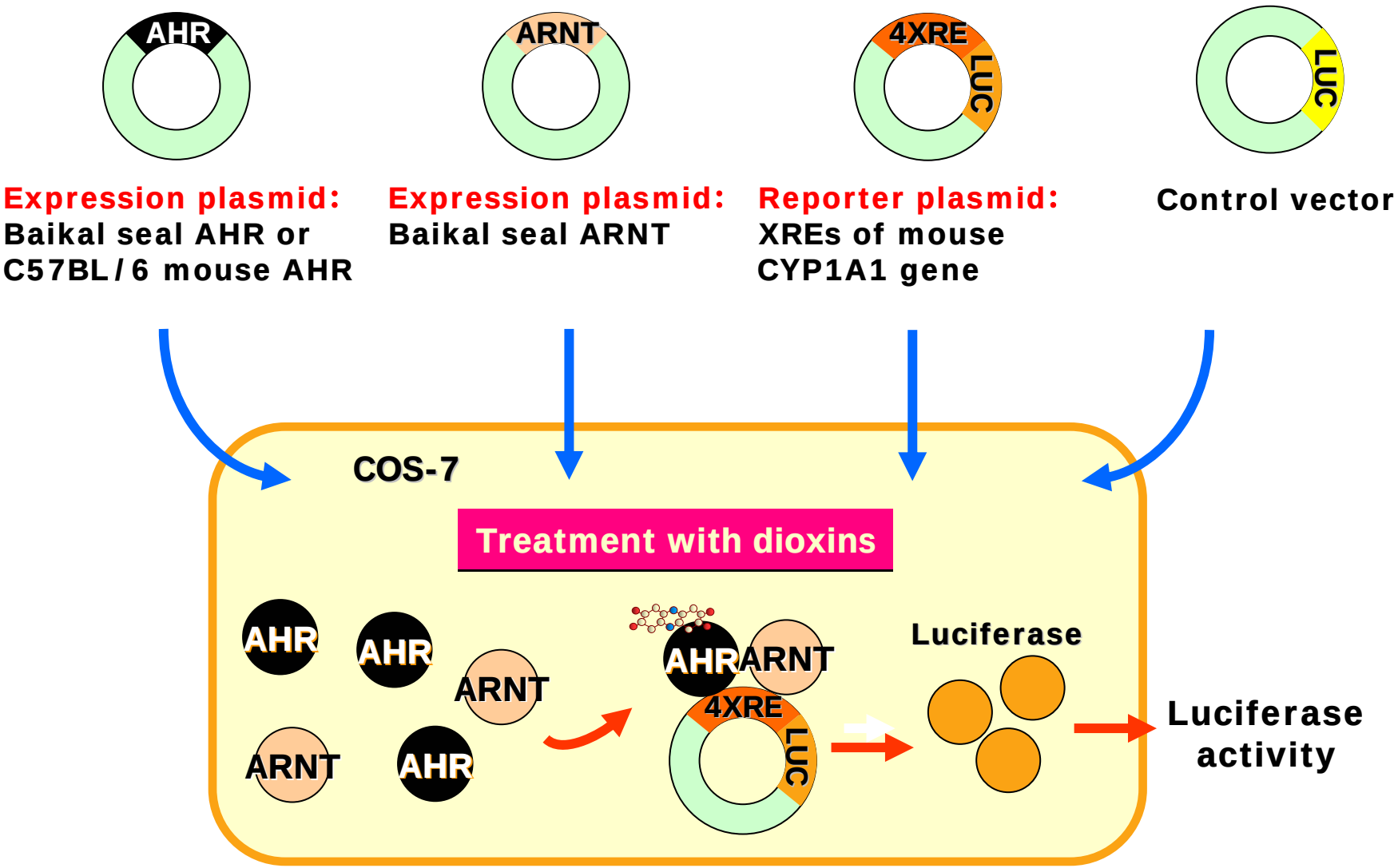
cDNA cloning of AHR, ARNT & CYP1A1/1A2/1B1 from Baikal seal

Kim *et al.*, *Mar. Environ. Res.*, 54, 285-289, 2002.

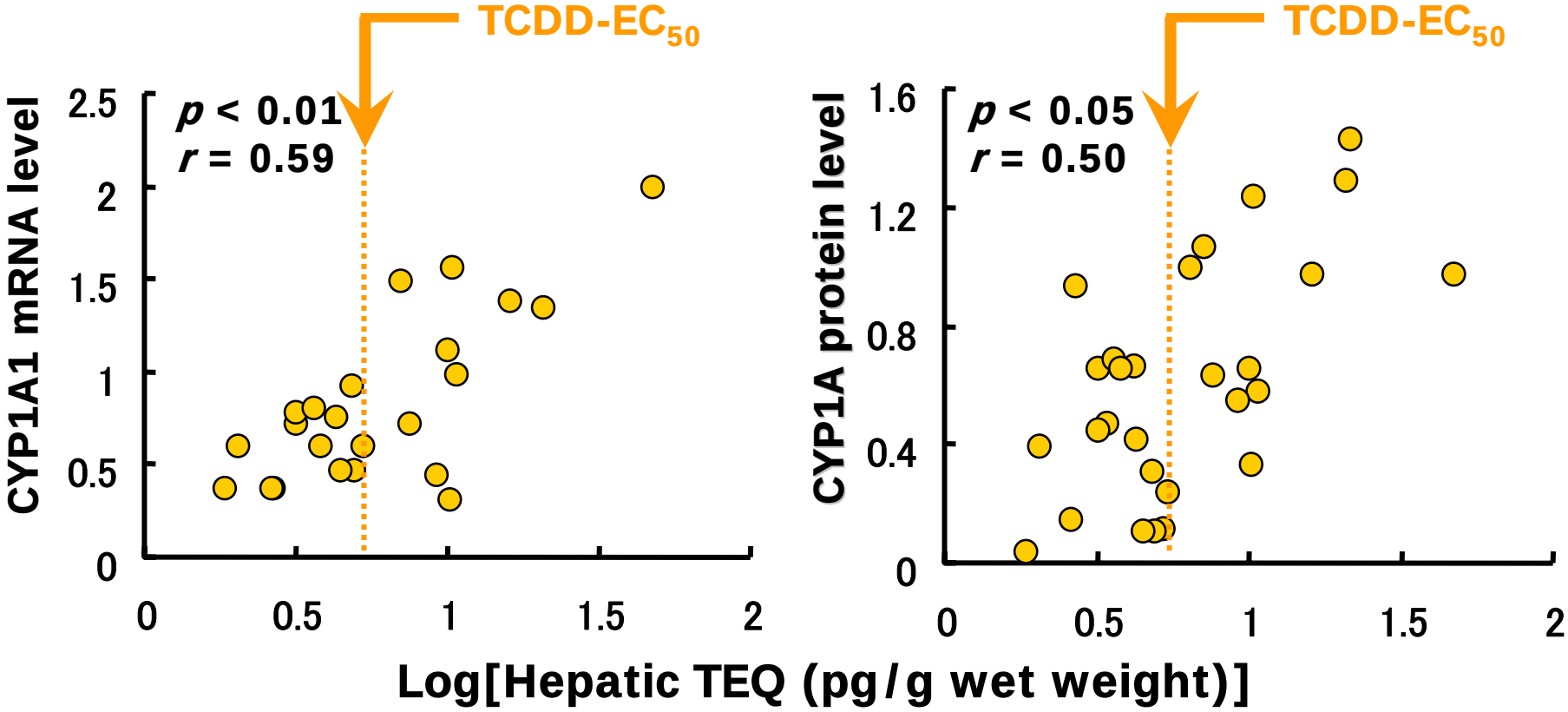
Kim *et al.*, *Comp. Biochem. Physiol. C*, 141, 281–291, 2005.

Hirakawa *et al.*, *Toxicol. Sci., 97, 318-335, 2007*.

Transactivation analysis of AHR: *in vitro* reporter gene assay



Relationships between hepatic TEQs and CYP1A expression levels in wild Baikal seal population



Hirakawa *et al., Toxicol. Sci.*, 97, 318-335, 2007

Our previous investigation showed significant positive correlations between hepatic TEQs and CYP1A1 levels in the wild Baikal seal population. This is in consistent with an indication from the *in vitro* Baikal seal AHR transactivation assay.

Conclusions

1. **Results derived from experiments focusing on AHRs of limited model species could not simply be extrapolated into AHR-mediated responses in wildlife.**

2. **Knowledge on species-specificity of AHR-mediated responses is necessary to assess the risk of dioxins in wildlife.**

3. **The *in vitro* reporter gene assay system that is constructed using expression vectors of AHRs from wildlife can potentially be a valuable tool for evaluating sensitivity to dioxins, and consequently for assessing the risk in target species.**

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