# PREDICTIONS ON GENES THAT CONTRIBUTE TO RESISTANCE OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) TOXICITY IN MICE AND RATS

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## 1. INTRODUCTION

TCDD is a chemical extremely toxic in some animal species [1]. However, there are marked differences in the sensitivity of various mammalian species and strains with respect to toxicity of TCDD. Male **young** guinea pigs with an 30-d $LD_{50}$ of ca. 1 µg/kg b.wt and **adult** Han Wistar (Kupio) rats with an $LD_{50}$ of more than 9600 µg/kg represent the two extremes [2,3]. The question of species differences is one of the central issues of the controversy that emerged on the validity of risk assessment strategies for TCDD and related compounds.

One of the most challenging issues facing toxicologists today is the identification of genes that contribute or are responsible for increased sensitivity or resistance to TCDD and related environmental chemicals [4]. It is assumed that most, if not all, toxic effects of TCDD are mediated through binding to the Ah receptor [5,6]. Recently GONZALEZ and his team produced an Ah receptor-deficient mouse by „knocking out" the gene that encodes the Ah receptor [7]. This mouse strain is relatively resistant to toxic effects of TCDD, such as enzyme induction and lethality [7]. It was found that the C57BL/6J mice, which possess the $Ah^{b-1}$ receptor [8], have a high binding affinity (e.g. low $K_d$ value) to TCDD and are very sensitive to toxicity of TCDD. The DBA/2J mice belong to a mouse strain which is relatively resistant to the toxicity of TCDD. They bear the $Ah^d$ receptor [8] which has a low binding affinity (high $K_d$ value) for TCDD [9]. However, recently DEVITO et al. [5] came to the conclusion that there is no obvious correlation between the binding affinity of TCDD to the Ah receptor and the species sensitivity to the lethal or other toxic effects of TCDD. Therefore, it was concluded by ROZMAN [10a], SAFE [10b], and DEVITO and BIRNBAUM [10c] that other factors may contribute to the resistance or sensitivity of mammals to TCDD toxicity.

One important factor which, beside the Ah receptor and pharmacokinetics [10c, 17], may modulate the TCDD toxicity seems to be total body fat (lipid) content of the animals [3], since a correlation has been found between toxicity ($LD_{50}$ ) of TCDD in different animals and their total body fat content [11]. That means that animals with a high fat content (e.g. **adult** hamsters)

are relatively resistant to the toxicity of TCDD, e.g. they have a high $LD_{50}$ .Therefore, in this respect genes, such as obese (*ob*), diabetes (*db*), yellow ($A^y$), and fatty (*fa*), which are responsible for obesity of mice and/or rats, are of great interest. The objective of this study was to look for genes using congenic mice and rat strains that affect the resistance or sensitivity of these mammals to TCDD toxicity and to make predictions regarding $LD_{50}$ values.

## 2. MATERIAL AND METHODS

The total body fat content of congenic strains of mice and rats **with single gene mutations** were taken from the literature. Their acute toxicity (30-d $LD_{50}$ in µg/kg b.wt.) was calculated from their total body fat content (TBF %) by equation (1) [3,11]:

$$\log LD_{50} = 5.30 * \log (TBF) - 3.22 \qquad (1)$$

## 3. RESULTS AND DISCUSSION

The predicted acute toxicity (30-d $LD_{50}$ ) of genetically obese C57BL/6J-*ob/ob* mice, yellow C57BL/6J-$A^y/a$ mice, and diabetic C57BL/KsJ-*db/db* mice were calculated and compared to lean wild type mice. These data with their body fat content, age, and the resistance factors are compiled in Tables 1-3. In Table 4 the predicted acute toxicity of TCDD

**TABLE 1: PREDICTED ACUTE TOXICITY (ORAL 30-DAYS $LD_{50}$) OF TCDD IN OBESE C57 BL/6J-*ob/ob* MICE IN COMPARISON TO LEAN C57 BL/6J-+/+ MICE**

| Mouse C 57 BL / 6J (Mutant) | Age (days) | Mean fat content[a] (%) | Predicted 30-d $LD_{50}$ [b] (µg/kg body wt.) | Resistance factor [c] |
|---|---|---|---|---|
| obese (*ob/ob*) | 6-16 | 15.2 | 1110 | 3 |
| lean (+/+) | 6-16 | 12.3 | 360 | |
| obese (*ob/ob*) | 18-22 | 16.5 | 1710 | 25 |
| lean (+/+) | 18-22 | 9.0 | 69 | |
| obese (*ob/ob*) | 28-32 | 18.9 | 3510 | 117 |
| lean (+/+) | 28-32 | 7.7 | 30 | |
| obese (*ob/ob*) | 45-55 | 29.3 | 35,840 [d] | 1710 |
| lean (+/+) | 45-55 | 7.2 | 20 | |
| obese (*ob/ob*) | >1 year | 59.1 | 1,480,000 [d] | 3060 |
| lean (+/+) | >1 year | 13.0 | 480 | |

a) The mean total body fat content data of the obese and lean mice are from HERBERG[12].

b) Calculated from equation (1).

c) Resistance factor = $\frac{LD_{50} \text{ of obese C 57 BL/6 J mouse}}{LD_{50} \text{ of lean C 57 BL / 6 J mouse}}$

d) It is unlikely that this 30-day $LD_{50}$ value of TCDD can be determined.

is presented in obese (*fa/fa*) Zucker rats in comparison to lean (*Fa/Fa*) Zucker rats. It was calculated that the C57BL/6J-*ob/ob* mice are between 3 and 3060 times more resistant (see Table 1), the genetically obese yellow C57BL/6J-$A^y/a$ mice are between 5 and 118 times more resistant (see Table 2), and the diabetic C57BL/KsJ-*db/db* mice are between 9 and 7526 times more resistant to acute toxicity of TCDD in comparison to the congenic wild-type mouse strain.

**TABLE 2: PREDICTED 30-DAYS $LD_{50}$ OF TCDD IN MALE AND FEMALE GENETICALLY OBESE YELLOW ($A^y/a$) AND LEAN NON-YELLOW ($a/a$) MICE**

| Mouse strain C57BL/6J | Sex (m/f) | Body weight (g) mean ± SEM | Age (days) | Total body fat[a] (%) | Reference | Predicted 30-days $LD_{50}$[b] (µg/kg b.wt.) | Resistance Factor[c] |
|---|---|---|---|---|---|---|---|
| $A^y/a$ [d] | m | 32.4 ± 0.6 | 135-140 | 36.4 | PLOCHER & POWLEY (1976) | 113,200 [f] | 5 |
| $a/a$ [d] | m | 25.1 ± 2.3 | 135-140 | 26.7 | | 21,900 [f] | |
| $A^y/a$ [d] | f | 33.7 ± 0.6 | 135-140 | 49.9 | PLOCHER & POWLEY (1976) | 602,500 [f] | 21 |
| $a/a$ [d] | f | 21.3 ± 1.1 | 135-140 | 28.2 | | 29,260 [f] | |
| $A^y/a$ [e] | m | 50.7 | 180 | 33.0 | FENTON & CHASE (1951) | 67,320 [f] | 42 |
| $a/a$ [e] | m | 34.0 | 180 | 16.3 | | 1,600 | |
| $A^y/a$ [e] | f | 49.0 | 180 | 35.9 | FENTON & CHASE (1951) | 105,200 [f] | 118 |
| $a/a$ [e] | f | 27.7 | 180 | 14.6 | | 890 | |

a) Fat (%) was calculated from the fat-amount data given by PLOCHER and POWLEY (1976) [13] or taken from FENTON & CHASE (1951)[14]

b) Calculated from equation (1).

c) Resistance Factor = $\frac{LD_{50} \text{ of obese yellow } (A^y/a) \text{ mice of the same sex and age}}{LD_{50} \text{ of lean non-yellow } (a/a) \text{ mice of the same sex and age}}$

d) Genetically obese yellow ($A^y/a$) and non-yellow ($a/a$) mice were bred by mating C57 BL/6J-$A^y/a$ females with a/a males [13].

e) Mice received diet No. 226 (30% casein, 17.5% sucrose, 5% corn oil, 42.5% hydrogenated cotton seed oil and 5% salt mixture; for more information see [14] ).

f) It is unlikely that this 30-day $LD_{50}$ value of TCDD can be determined.

**TABLE 3:** PREDICTED ACUTE TOXICITY (ORAL 30-DAY $LD_{50}$) OF TCDD IN **DIABETIC** (C57 BL/KsJ-*db/db*) MICE IN COMPARISON TO LEAN (C57 BL/KsJ-+/+) MICE

| Mouse C57 BL/KsJ (Mutant) | Age (days) | Mean fat content[a] (%) | Predicted 30-d $LD_{50}$[b] (µg/kg b.wt.) | Resistance factor [c] |
|---|---|---|---|---|
| KsJ-db/*db* | 6-16 | 16.2 | 1550 | 9 |
| KsJ-+/+ | 6-16 | 10.8 | 180 | |
| KsJ-*db/db* | 18-22 | 17.9 | 2630 | 23 |
| KsJ-+/+ | 18-22 | 9.9 | 114 | |
| KsJ-*db/db* | 28-32 | 16.7 | 1820 | 433 |
| KsJ-+/+ | 28-32 | 5.3 | 4.2 | |
| KsJ-*db/db* | 45-55 | 32.7 | 64,140[d] | 4823 |
| KsJ-+/+ | 45-55 | 6.6 | 13.3 | |
| KsJ-*db/db* | >1 year | 52.8 | 812,756[d] | 7526 |
| KsJ-+/+ | >1 year | 9.8 | 108 | |

a) The mean total body fat content data of diabetic C57 BL/KsJ-*db/db* and lean mice were taken from HERBERG (1991) [12].

b) Calculated from equation (1).

c) Resistance Factor = $\frac{LD_{50} \text{ of diabetic C57 BL/KsJ-}db/db \text{ mice}}{LD_{50} \text{ of lean C57 BL/KsJ-+/+ mice}}$

d) It is unlikely that a 30-d $LD_{50}$ of TCDD can be determined with such a high dose of TCDD.

**Tabelle 4:** PREDICTED ACUTE TOXICITY (30-DAY $LD_{50}$) OF TCDD IN **FATTY**(*fa/fa*) **ZUCKER RATS** IN COMPARISON TO LEAN (*Fa/Fa*) ZUCKER RATS

| Rat strain (mutant) | Age (days) | Body weight (g) | Mean fat content (TBF %) | 30-d $LD_{50}$ (µg/kg) | Resistance factor |
|---|---|---|---|---|---|
| Zucker rat (*fa/fa*) | 31 | 70 | 17.5 [a] | 2230 | |
| Zucker rat (*Fa/Fa*) | 31 | 62 | 6.1 [a] | 40 | 56 |
| Zucker rat (*fa/fa*) | 150 | 543 | 39.9 [b] | 184,140 [c] | |
| Zucker rat (*Fa/Fa*) | 150 | 396 | 9.3 [b] | 82 | 2250 |

a) BELL and STERN (1977) [15].

b) TRIMBLE et al. (1980) [16].

c) It is unlikely that a 30-d $LD_{50}$ value can be determined experimentally.

## 4. CONCLUSIONS

From the genetic background it is known that the C57BL/6J and the C57BL/KsJ mice carry the $Ah^{b-1}$ receptor which binds TCDD with high affinity. In case the *Ahr* gene alone would be responsible for the sensitivity or resistance to toxic effects of TCDD, the congenic mouse strains presented in Table 1, 2, and 3 should have the same sensitivity regarding to the toxicity of TCDD. However, according to our predictions the mouse strains homozygous for *ob* and *db* gene, or heterozygous for the $A^y$ gene, respectively, should be more resistant to the toxicity of TCDD in comparison to congenic lean wild-type mouse strain.

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## 5. REFERENCES

1) BIRNBAUM, L.S. (1994). The mechanism of dioxin toxicity: Relationship to risk assessment. Environ. Health Perspect. *102*, Supplement 9, 157-167.

2) POHJANVIRTA, R. and TUOMISTO, J. (1994). Short-term toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in laboratory animals: effects, mechanisms, and animal models. Pharmacol. Rev. *46* (4), 483-549.

3) GEYER, H., SCHEUNERT, I., RAPP, K., GEBEFÜGI, I., STEINBERG, C., and KETTRUP, A. (1993). The relevance of fat content in toxicity of lipophilic chemicals to terrestrial animals with special reference to dieldrin and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Ecotoxicol. Environ. Saf. *26*, 45-60.

4) PUGA, A., MICKA, J., CHANG, C.-Y., LIANG, H.-C., and NEBERT, D.W. (1995). Role of molecular biology in risk assessment. In:"*Biological Reactive Intermediates V: Basic Mechanistic Research in Toxicology and Human Risk Assessment.*" Plenum Publishing Corp., New York, N.Y. (in press).

5) DEVITO, M.J., BIRNBAUM, L.S., FARLAND, W.H. and GASIEWICZ, T.A. (1995). Comparisons of estimated human body burdens of dioxinlike chemicals and TCDD body burdens in experimentally exposed animals. Environ. Health Perspect. *103*, 820-831.

6) GALLO, M.J., SCHEUPLEIN, R.J., VAN DER HEIDEN, K.A. (Eds.) (1991). „*Biological Basis for Risk Assessment of Dioxins and Related Compounds.*" Banbury Report No. 35. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

7) FERNANDEZ-SALGUERO, P., PINEAU, T., HILBERT, M., MCPHAIL, T., LEE, S.S.T., KIMURA, S., NEBERT, D.W., RUDIFKOFF, S., WARD, M., and GONZALEZ, F.J. (1995). Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. Science *268*, 722-726.

8) POLAND, A., PALEN, D., and GLOVER, E. (1994). Analysis of the four alleles of the murine Ah receptor. Mol. Pharmacol. *46*, 915-921.

9) OKEY, A.B., RIDDICK, D.S., and HARPER, A. (1994). The Ah receptor: Mediator of the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds. Toxicol. Lett. *70*, 1-22.

10a) ROZMAN, K. (1989). A critical view of the mechanism(s) of toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: Implications for human safety assessment. Dermatosen Beruf Umwelt *37*, 81-92.

10b) SAFE, S.H. (1990). Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit. Rev. Toxicol. *21*, 51-88.

10c) DEVITO, M.J. and BIRNBAUM L.S. (1995). The importance of pharmacokinetics when estimating the relative potency of 2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzoluran. Fund. Appl. Toxicol. 24, 145-148.

11) GEYER, H.J., SCHEUNERT, I., RAPP, K., KETTRUP, A., KORTE, F., GREIM, H. and ROZMAN, K. (1990). Correlation between acute toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and total body fat content in mammals. Toxicology *65*, 97-107.

12) HERBERG, L. (1991). Interrelationships between obesity and diabetes. *„Proceedings of the Nutrition Society". Symposium on 'Dietary Factors and Therapy of Diabetes'*. 19-21 June 1991 at The University of Ulster at Coleraine, Northern Ireland. *50*, 605-617.

13) PLOCHER, T.A., and POWLEY, T. (1976). Effect of hypophysectomy on weight gain and body composition in the genetically obese yellow ($A^y/a$) mouse. Metabolism *25*, 593-602.

14) FENTON, R.F., and CHASE, H.B. (1951). Effect of diet on obesity of yellow mice in inbred lines. Proc. Soc. Exp. Biol. Med. *77*, 420-422.

15) BELL, G.E., and STERN, J.S. (1977). Evaluation of body composition of young obese and lean Zucker rats. Growth 41, 63-80.

16) TRIMBLE, E.R., HERBERG, L., and RENOLD, A.E. (1980). Hypersecretion of pancreatic somatostatin in the obese Zucker rat. Effect of food restriction and age. Diabetes *29*, 889-894.

17) VAN DEN BERG, M., DE JONGH, J. POIGER, H., and OLSON, J., R. (1994). The toxicokinetics and metabolism of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) and their relevance for toxicity. Crit. Rev. Toxicol. 24(1) 1-74.