Accumulated TCDD is redistributed during a common infection (Coxsackievirus B3) in the mouse

Eva Funseth[1)], Anders Wicklund-Glynn[2,3)], Göran Friman[1)] and Nils-Gunnar Ilbäck[1,2)]
1) Department of Infectious Diseases and Clinical Microbiology, Uppsala University Hospital, 751 85 Uppsala, Sweden
2) Toxicology Division, National Food Administration, P.O. Box 622, 751 26 Uppsala
3) Department of Environmental Toxicology, Uppsala University, Norbyv. 18A, 752 36 Uppsala

Abstract
The redistribution during infection of accumulated 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was studied in a mouse model using a common human virus, Coxsackievirus B3 (CB3) adapted to the mouse. The day of virus inoculation was designated day 0. Adult male A/J-mice were injected i.p. with 1 μCi ^{3}H-TCDD, corresponding to a dose of 0,5μg TCDD/kg, on day -1. On day 0 half of the mice were inoculated with CB3 virus and the other half served as uninfected controls. Thymus, spleen, heart, pancreas, liver and white adipose tissue were collected on day 0 (prior to infection), and on days 4 and 7 of the infection. The ^{3}H-TCDD levels of the tissues were determined by liquid scintillation techniques. In the uninfected controls the overall picture indicated that most of the administered TCDD dose had been taken up and distributed before the first sampling point. Our results show a redistribution of TCDD as a result of the infection. In two of the most important target organs for CB3 virus, i.e. the pancreas and heart, the ratio between TCDD concentration found in infected mice and in controls peaked concurrent with the development of inflammatory lesions in the infected group. Similarly, the TCDD levels in the thymus of infected mice increased three-fold between day 0 and 7 to an estimated 0.5 pmol TCDD/g tissue, a concentration where suppression of T cell proliferation has earlier been found. Interference of TCDD with T cell function in the thymus and host responses in the target organs of the CB3 virus may alter the course of the infection. Considering that children often experience frequent infections during infancy, an infection-induced redistribution of TCDD might become clinically relevant in infants. There is no reason to believe that the redistribution pattern shown in the present study is unique to TCDD or to the presently used microorganism. Thus, the possible implications of infection-associated tissue redistribution of toxicants should be considered in risk assessment.

Introduction
Host resistance studies indicate that exposure to TCDD and related compounds may increase the host's susceptibility to viral and parasitic, as well as to bacterial pathogens[1)]. We have found that Coxsackievirus B3 (CB3) infection in mice may alter the tissue distribution of environmental pollutants such as TCDD when exposure occurs during ongoing infection[2-4)]. Coxsackie B (CB) viruses are common infectious agents and virtually all humans encounter several serotypes of these viruses during their life time[5)]. Usually these infections pass unrecognized or give rise to only mild upper respiratory tract or gastrointestinal symptoms. However, sometimes CB3 causes myocarditis, pancreatitis or meningoencephalitis. Murine models of CB3 infection have been developed where myocarditis is a prominent feature, with a pathogenesis that closely resembles that of CB myocarditis in humans[5)]. In healthy individuals of most species the major sites of TCDD accumulation are the liver and adipose tissue[6)]. During infections energy depots in the body are mobilized to meet nutritional requirements and to supply building blocks necessary for tissue repair and synthesis of host defence constituents[7,8)]. Our hypothesis is that during infections, toxicants previously accumulated as a result of long-time exposure may be released from the liver and/or adipose tissue, resulting in a temporary increased systemic exposure and potential target organ toxicity. We have studied whether a common human viral infection (CB3), adapted to a mouse model, causes tissue redistribution of TCDD from its principal accumulation sites in the liver and adipose tissue to other organs.

Experimental Methods

Material

Mice. Adult male A/J-mice were randomized into groups of similar initial mean body weight and acclimatized for one week before treatment. The mice were housed five per cage at 23±1°C on a 12-hour light/dark cycle with free access to food and water.

Virus. Each mouse was inoculated i.p. with $2x10^4$ plaque forming units of a human myocarditic Coxsackievirus type B3 adapted to the mouse[5]. The CB3 virus was dosed in order to achieve a mortality and size of inflammatory and necrotic lesions in the myocardium corresponding to that previously recorded in CB3-infected male A/J mice[9].

TCDD. 2,3,7,8-tetrachloro[1,6-^{3}H]dibenzo-p-dioxin with a specific activity of 24,4 Ci/mmol (96% radioactive purity) was used. The solvent (toluene) was removed under a stream of nitrogen gas. The tritiated TCDD was then dissolved in sterile corn oil to the appropriate concentration.

Treatment and tissue sampling procedures

TCDD treatment and infection. The day of virus inoculation was designated day 0. On day -1 all mice received 1μCi of ^{3}H-TCDD i.p., corresponding to a dose of 0,5μg TCDD/kg. Before infection on day 0 one group of mice (n=8) was sacrificed, serving as "day 0 controls". Of the remaining mice one subgroup (n=21) was inoculated i.p. with CB3 virus, while the other subgroup (n=16) served as a TCDD-exposed, non infected control.

Tissue sampling. Infected mice and uninfected controls were sacrificed on days 4 and 7 post inoculation (p. inoc.) for tissue sampling. Mice were anaesthesized with Hypnorm/Dormicum and thymus, spleen, heart, pancreas, liver and white adipose tissue (WAT) were dissected, weighed and prepared for liquid scintillation.

Liquid scintillation counting

The ^{3}H-TCDD-content of the tissues was determined by means of standard liquid scintillation techniques. Coarse-ground tissue samples were digested in alkaline tissue solubilizer. Blood-rich samples were bleached with 30% H_2O_2. Finally, scintillation fluid was added and the samples were analyzed in a liquid scintillation counter.

Statistical analysis

The effects of infection on ^{3}H-TCDD tissue redistribution were calculated using Student´s t-test, comparing the uninfected control group and the CB3 infected group.

Results and Discussion

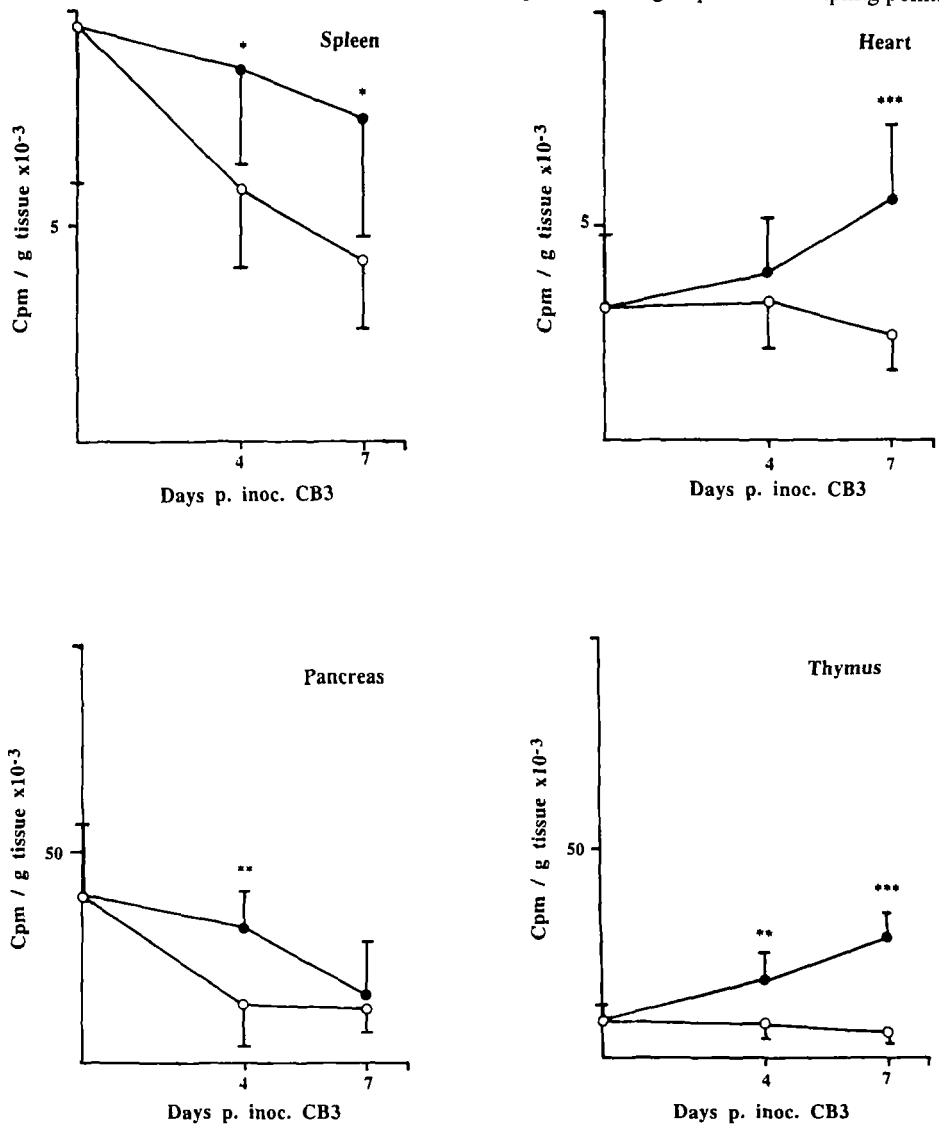
The present study shows that a common viral infection, CB3 infection, may cause redistribution of a previously accumulated toxicant (TCDD). This may result in a peak of systemic exposure potentially affecting the outcome of the disease, as well as increasing TCDD levels in target organs to concentrations which might induce toxic insult.

Among the studied organs, the TCDD tissue concentration was highest in the liver and white adipose tissue (WAT) of infected as well as uninfected mice. In the liver the TCDD concentration peaked (n.s.) on day 4 in both groups (data not shown). In adipose tissue the TCDD concentration increased almost four-fold over time in infected mice to a maximum of 162218±26855 cpm/g tissue (mean±S.D.) recorded on day 7, whereas it remained at a relatively stable level in the controls during the study period (70396±15537 on day 7).

Coxsackie B3 virus shows tissue specific tropism for the heart and pancreas[5]. In both these organs the TCDD concentration seemed to peak concurrent with the development of inflammatory and necrotic lesions (Figure 1). In the pancreas, the rapid decrease in TCDD concentration which was seen in the controls, did not occur in infected mice, who had twice as high TCDD concentration on day 4 as the controls, decreasing until day 7 to levels found in the controls. The TCDD concentration in the heart of infected mice peaked on day 7, when myocardial inflammation is most prominent, and was by then twice as high as in the controls. In the immunologically active organs, i.e. thymus and spleen, the concentration was higher in the infected mice than in uninfected controls at both sampling points (Figure

1). The TCDD concentration in the thymus of infected mice increased three-fold between days 0 and 7. This can partly be explained by the normally occurring outflow of thymocytes during infection which diminish thymic weight considerably. The thymic TCDD concentration in the infected mice reached 0,5 pmol TCDD/g tissue which is above the levels known to cause suppressed T-cell function in adult mice[10]. In infected mice the TCDD concentration in the spleen showed a similar tendency of a decrease over time as in the uninfected controls. However, it was significantly higher, at both sampling points, during infection.

Figure 1. TCDD concentration in the thymus, spleen, heart and pancreas of healthy controls (O) and CB3-infected (●) adult male A/J mice on day 0, 4 and 7 p. inoc.; n=8/group at each sampling point.



Values are given as means ± S.D. Statistically significant differences between control and infected groups are denoted by asterisks (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).

Our hypothesis was that the infection-induced redistribution of TCDD might be an event accompanying the mobilization of energy depots in the body, which generally occurs to meet the host's nutritional requirements for combating an infection[7,8]. Body weight decreased, as a result of the infection. Although it was not possible to measure the total adipose tissue mass, this is also known to decrease during infection. The considerably increased TCDD concentration in WAT recorded in infected mice, indicates that some of the stored TCDD was redistributed within this tissue. However, it is still likely that part of the TCDD stored in WAT was released to the circulation. In the other major TCDD accumulation site, i.e. the liver, TCDD concentration did not differ significantly between the groups. Infection is known to suppress P450-mediated metabolism, possibly through cytokines that are induced by viruses[11]. However, as A/J mice are relatively resistant to the AhR mediated enzyme-inducing properties of TCDD, infection-induced suppression of P450-mediated metabolic processes did probably not influence distribution to any higher degree in the present study[12-14]. Infection may cause lipid accumulation in certain tissues, such as the heart, through mechanisms that are not fully understood[15]. Furthermore it has been suggested that macrophages actively transport lipids into inflammatory lesions in similarity to the suggested mechanisms for atherosclerotic plaque formation[15]. This might be of importance for the observed distribution to the pancreas and the heart. At low dose exposure, TCDD distribution is dependent mainly on the lipid content of different tissues[16,17]. Environmental and nutritional factors might interact with host responses in target organs of infection, resulting in more severe disease[18-20]. Furthermore, an infection-induced increase in TCDD concentration in the thymus may alter T cell function through effects on thymic epithelial cells necessary for supporting T cell development and proliferation[21]. Interference with T cell function might alter the course of CB3 myocarditis. In athymic mice, CB3 virus may persist for several months after the initial infection, in contrast to CB3 myocarditis in euthymic mice, where virus clearance is complete by two weeks post inoculation[22].

The possible relevance of an infection-induced redistribution becomes evident considering that children often experience frequent infections during infancy. Before reaching one month of age 12.6% of neonates contract a Coxsackievirus infection, and also pregnant women show increased susceptibility to these viruses[5,23,24]. This is the first study to show that a common infection causes redistribution of a previously accumulated environmental pollutant. There is no reason to believe that the redistribution pattern shown in the present study is unique to TCDD or to the presently used microorganism. Thus, the possible implications of infection-associated tissue redistribution of toxicants should be considered in risk assessment.

Acknowledgements
The project was supported by grants from the Swedish National Environment Protection Agency.

Literature Cited

(1) Kerkvliet, N.I. *Environ. Health Perspect.* **1995**, 103, 47-53.
(2) Funseth, E.; Ilbäck, N.-G. *Toxicology* **1994**, 90, 29-38.
(3) Ilbäck, N.-G.; Fohlman, J.; Friman, G. *Toxicol. Appl. Pharmacol.* **1992**, 114, 166-170.
(4) Ilbäck, N.-G.; Fohlman, J.; Friman, G.; Wicklund-Glynn, A. *Toxicology* **1992**, 71, 193-202.
(5) Woodruff, J.F. *Am. J. Pathol.* **1980**, 101, 425-483.
(6) Neal, R.A.; Olson, J.R.; Gasiewicz, T.A.; Geiger, L.E. *Drug Metab. Rev.* **1982**, 13, 355-385.
(7) Ilbäck, N.-G.; Friman, G.; Squibb, R.L.; Johnson, A.J.; Balentine, .D.A.; Beisel, W.R. *Acta Pathol. Microbiol. Immunol. Scand.* **1984**, 92, 195-204.
(8) Beisel, W.R. *Am. J. Clin. Nutr.*, **1982**, 35, 417-468.
(9) Fohlman, J.; Pauksen, K.; Hyypiää, T.; Eggertsen, G.; Ehrnst, A.; Ilbäck, N.-G.; Friman, G. *Circulation* **1996**, 94, 2254-2259.
(10) Luebke R.W.; Copeland, C.B.; Diliberto, J.J.; Akubue, P.I.; Andrews, D.L.; Riddle, M.M.; Williams, W.C.; Birnbaum, L.S. *Toxicol. Appl. Pharmacol.* **1994**, 12, 7-16.
(11) Renton, K.W.; Knickle, L.C. *Can. J. Physiol. Pharmacol.* **1990**, 68, 777-781.
(12) Birnbaum, L.S. *Drug Metab. Dispos.* **1986**, 14, 34-40.
(13) Nebert, D.W.; Jensen, N.M. *Genetics* **1982**, 100, 79-87.

(14) Smith, A.G.; Francis, J.E. *Biochem. J.* **1987**, 246, 221-226.
(15) Ilbäck, N.-G.; Mohammed, A.; Fohlman, J.; Friman, G. *Am. J. Pathol.* **1990**, 136, 159-167.
(16) Diliberto, J.J.; Akubue, P.I.; Luebke, R.W.; Birnbaum, L.S. *Toxicol. Appl. Pharmacol.* **1995**, 130, 197-208.
(17) Leung, H.-W.; Wendling, J.M.; Orth, R.; Hileman, F.; Paustenbach, D.J. *Toxicol. Lett.* **1990**, 50, 275-282.
(18) Beck, M.; Kolbeck, P.C.; Qing, S.; Rohr, L.H.; Morris, V.C.; Levander, M.A. *J. Infect. Dis.* **1994**, 170, 351-357.
(19) Ilbäck, N.-G.; Wesslén, L.; Fohlman, J.; Friman, G. *Toxicol. Lett.* **1996**, 89, 19-28.
(20) Ilbäck, N.-G.; Fohlman, J.; Friman, G. *J. Trace Elem. Exp. Med.* **1989**, 2, 257-266.
(21) de Waal, E.J.; Schuurman, H.-J.; Loeber, J.G.; van Loveren, H.; Vos, J.G. *Toxicol. Appl. Pharmacol.* **1992**, 115, 80-88.
(22) Sato, S.; Tsutsumi, R.; Burke, A.; Carlson, G.; Porro, V.; Seko, Y.; Okumura, K.; Kawana, R.; Virmani, R. *J. Gen. Virol.* **1994**, 75, 2911-2924.
(23) Cherry, J.D. *Infectious Diseases of the Fetus and Newborn Infant*; W.B. Saunders Comp.; Philadelphia, **1982**; p. 290.
(24) Morag, A.; Ogra, P.L. *Nelson Textbook of Pediatrics*; W.B. Saunders Comp.; Philadelphia, **1996**; p. 875.