The poisoning of Victor Yushchenko

Published Online August 5, 2009 DOI:10.1016/S0140-6736(09)61027-8

See Articles page 1179

To understand the events described by Oliver Sorg and colleagues[1] in *The Lancet* today, about the poisoning of Victor Yushchenko, we need to be aware of the role that national identity plays in Ukrainian politics. Does Ukraine's future lie within a grouping of states clustered around a resurgent Russia or does it lie to the west, perhaps within an enlarged European Union? Ukraine is divided geographically, with a majority of individuals living in the east, including a substantial minority of ethnic Russians, who favour a resurgent Russia, while most Ukrainians living in the west favour stronger links with the rest of Europe.[2]

Leonid Kravchuk, who in 1991 became the first President of the newly independent Ukraine, sought to position his country somewhere in the middle, asserting Ukrainian sovereignty while breaking many of the former ties with Russia. This positioning was too much for many people living in east Ukraine and, in the 1994 presidential election, Kravchuk was ousted by Leonid Kuchma whose campaign included a commitment to restore economic ties with Russia.

By 2004, Kuchma had served the maximum two terms permitted by the constitution and had to stand down. He was, by this time, deeply unpopular and his reputation had been battered by allegations of corruption and the appearance of tape-recorded conversations that seemed to implicate him in the murder of a prominent journalist 4 years previously.[3]

The election was mired in controversy from the start. 26 candidates stood but only two had a realistic prospect of success. Victor Yanukovych was the incumbent Prime Minister and Kuchma's preference. As the representative of the ruling Party of the Regions, he stood on a platform of strengthened links with Russia. His main opponent was Yushchenko, who had also served as Prime Minister under Kuchma but had been ousted after losing a confidence vote in parliament in 2001, instigated by a combination of communists and groups linked to oligarchs who opposed his economic reforms. He went on to form the Our Ukraine political coalition, which became the largest party in the 2002 parliamentary elections, although falling short of an absolute majority. In the presidential election, Yushchenko was standing as an independent opponent, advocating a combination of Ukrainian nationalism with stronger ties to the European Union and membership of the North Atlantic Treaty Organization.

Yanukovych's campaign benefited from a virtual monopoly of coverage in the mainstream media. Most television channels were controlled by the state or by oligarchs, whereas the only channel seen as pro-Yushchenko was blocked in parts of the country at various times.[4] The campaign became deeply unpleasant, even including accusations that Yushchenko, whose father had been a prisoner in Auschwitz, was a Nazi. Yushchenko, by contrast, was energetically campaigning in rallies in which he was able to present his message to Ukrainians directly, and was tapping into a groundswell of popular discontent with the regime in power.

The first round of voting was to be held at the end of October but, just over a month earlier, soon after a dinner with senior officials of the Ukrainian security services, Yushchenko became seriously ill, with symptoms of acute pancreatitis. He was admitted to hospital in Vienna, Austria, where he made an initial recovery. His condition showed many unusual features but the cause remained a mystery and he returned to the campaign. 3 weeks later, however, his condition deteriorated and he developed a severe disfiguring rash. The late John Henry,[5] a British toxicologist, suggested dioxin poisoning, a diagnosis confirmed by investigations.[6]

Yushchenko returned to the campaign, although still seriously ill. The first round of voting produced no overall winner, with each of the two main candidates gaining just fewer than 40% of the votes. A second round in November gave victory to Yanukovych, with 49·4% of the votes, compared with Yushchenko's 46·7%. The regional voting pattern confirmed that the country was clearly divided between east and west. However, widespread electoral fraud was obvious, with many reports of ballot stuffing, intimidation of voters, and falsification of electoral rolls.[7] The situation rapidly became internationalised, with Russia and some of its neighbours rushing to congratulate Yanukovych on his election win whereas the European Union and USA refused to recognise the result. Within Ukraine, the result led to mass demonstrations, with most individuals in favour of Yushchenko. The Orange Revolution took

Corbis

Agent Orange
US air-force planes spraying Agent Orange near Saigon along the Cambodian border (Oct 14, 1968). Agent Orange (named after the orange barrels it came in) was a herbicide and defoliant that also contained dioxins, the poison that Victor Yushchenko was exposed to.

its name from the colour of his campaign material. Political deadlock ensued until, in early December, the Supreme Court ordered a rerun of the second round. With voting now under intense international scrutiny, Yushchenko emerged with an absolute majority and was inaugurated as President in January, 2005.

So who poisoned him? The obvious suspects are those members of the security service present at the dinner just before he fell ill, yet during the protests in December they and their colleagues gave covert support to Yushchenko, pre-empting a planned crack-down by Interior Ministry troops.[8] Unfortunately for those seeking an answer, there were many people, within Ukraine and outside it, who had a motive. We might never know and, as Sorg and colleagues note, had Yushchenko died at the time, as he might easily have done, we would probably never even have known that he had been poisoned.

Martin McKee
London School of Hygiene and Tropical Medicine,
London WC1E 7HT, UK
martin.mckee@lshtm.ac.uk

I declare that I have no conflicts of interest.

1 Sorg O, Zennegg M, Schmid P, et al. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) poisoning in Victor Yushchenko: identification and measurement of TCDD metabolites. *Lancet* 2009; published online Aug 5. DOI:10.1016/S0140-6736(09)60912-0.
2 Wilson A. The Ukrainians: unexpected nation. New Haven: Yale University Press, 2000.
3 BBC News. Kuchma to face Gongadze questions, March 9, 2005. http://news.bbc.co.uk/1/hi/world/europe/4333667.stm (accessed May 14, 2009).
4 Maksymiuk J. Analysis: Ukrainian media not playing fair in presidential campaign. Radio Free Europe http://www.media.com.pl/institute/ukraine/archive_art87.htm (accessed May 14, 2009).
5 Watts G. John Henry. *Lancet* 2007; **370:** 214.
6 BBC News. Yushchenko and the poison theory. Dec 11, 2004. http://news.bbc.co.uk/2/hi/health/4041321.stm (accessed May 14, 2009).
7 OSCE. Ukraine presidential election 31 October, 21 November and 26 December 2004. 2005. http://www.osce.org/documents/odihr/2005/05/14224_en.pdf (accessed 13 July, 2009).
8 Chivers CJ. How top spies in Ukraine changed the nation's path. *NY Tim* Jan 17, 2005.

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) poisoning in Victor Yushchenko: identification and measurement of TCDD metabolites

O Sorg, M Zennegg, P Schmid, R Fedosyuk, R Valikhnovskyi, O Gaide, V Kniazevych, J-H Saurat

Summary

Background 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has a long half-life of 5–10 years in human beings as a result of its high lipophilicity, and little or no metabolism. We monitored TCDD, its form, distribution, and elimination in Victor Yushchenko after he presented with severe poisoning.

Methods In late December, 2004, a patient presented with TCDD poisoning; the levels in his blood serum (108000 pg/g lipid weight) were more than 50000-fold greater than those in the general population. We identified TCDD and its metabolites, and monitored their levels for 3 years using gas chromatography and high-resolution mass spectrometry samples of blood serum, adipose tissue, faeces, skin, urine, and sweat, after they were extracted and cleaned with different organic solvents.

Findings The amount of unmodified TCDD in the samples that were analysed accounted for about 60% of TCDD eliminated from the body during the same period. Two TCDD metabolites—2,3,7-trichloro-8-hydroxydibenzo-p-dioxin and 1,3,7,8-tetrachloro-2-hydroxydibenzo-p-dioxin—were identified in the faeces, blood serum, and urine. The faeces contained the highest concentration of TCDD metabolites, and were the main route of elimination. Altogether, the different routes of elimination of TCDD and its metabolites accounted for 98% of the loss of the toxin from the body. The half-life of TCDD in our patient was 15·4 months.

Interpretation This case of poisoning with TCDD suggests that the design of methods for routine assessment of TCDD metabolites in human beings should be a main aim of TCDD research in the metabolomic era.

Funding University of Geneva Dermatology Fund, and Swiss Centre for Applied Human Toxicology.

Lancet 2009; 374: 1179–85
Published Online August 5, 2009
DOI:10.1016/S0140-6736(09)60912-0

See Comment page 1131

Dermato-Toxicology, Swiss Centre for Applied Human Toxicology, and Department of Dermatology, University Hospital, Geneva, Switzerland (O Sorg PhD, O Gaide MD, Prof J-H Saurat MD); **EMPA, Swiss Federal Laboratories for Materials Testing and Research, Dübendorf, Switzerland** (M Zennegg PhD, P Schmid PhD); **and Feofania Hospital, Kiev, Ukraine** (R Fedosyuk MD, R Valikhnovskyi MD, V Kniazevych MD)

Correspondence to:
Prof J-H Saurat, Dermato-Toxicology, Swiss Centre for Applied Human Toxicology, and Department of Dermatology, University Hospital, 4 rue Gabrielle Peret-Gentil, 1211 Geneva 4, Switzerland
Jean.Saurat@unige.ch

Introduction

"If there is no poison, there cannot be poisoning, and there was no trace of it whatsoever".[1] This statement shows the prevailing geopolitical and juridicial context that had impaired the scientific investigation to find out whether Victor Yushchenko, a candidate for the presidential election in Ukraine (figure 1), had been poisoned in 2004. While he was campaigning for the election, he suddenly became severely ill (figure 2) as a result of being poisoned during a dinner in Kiev on Sept 5, 2004.[2,3] However, identification of the poison—pure dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin [TCDD])—was delayed from Sept 5, 2004, until late December, 2004, because the presence of TCDD is not routinely investigated in medical practice in a patient with signs of acute poisoning. Therefore whether forensic investigators would have detected the poison in Victor Yushchenko had he died soon after the intoxication is unknown.

TCDD is the most potent member of a group of polyhalogenated aromatic hydrocarbons that includes polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls.[4,5] These lipophilic compounds diffuse freely across cell membranes, and exert their pleiotropic biological effects by binding to the intracellular aromatic hydrocarbon receptor.[6,7] Their toxic effects, particularly those of TCDD, are caused by their high affinity for this receptor, and by their long elimination half-lifes. Because polyhalogenated aromatic hydrocarbons are lipophilic, they accumulate in the lipids in tissues on a physical basis by simple partitioning; this process accounts for their slow elimination in the faeces. Only 17 (including TCDD) of 210 possible PCDDs and PCDFs have chlorine substituents at the lateral positions—ie, carbons 2, 3, 7, and 8, therefore preventing or greatly slowing their bioconversion to polar metabolites during oxidation by the phase I and phase II enzymes. An efficient bioconversion by enzymatic oxidation can take place when two adjacent hydrogen atoms are available, which is not the case when the lateral positions have chlorine substituents.[8–10] Although the metabolism of PCDFs can be induced by TCDD or by themselves,[11,12] TCDD has not been shown to induce its own metabolism.[13] The cytochrome P450 (CYP) monooxygenases CYP1A1, CYP1A2, and CYP1B1 have been shown to be substantially induced in human beings,[14,15] but metabolites of TCDD have not been clearly shown so far. The expected half-life of TCDD ranges from less than 5 years in individuals exposed to high levels—ie, more than 10000 pg/g lipid weight of TCDD in the blood serum—to more than 10 years in those exposed to less than 50 pg/g lipid weight.[16]

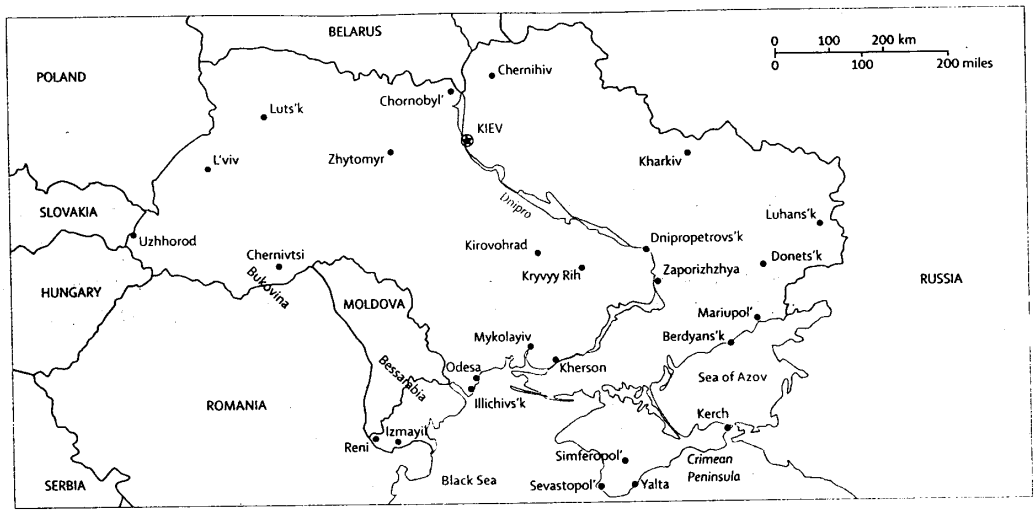


Figure 1: Map of Ukraine

In late December, 2004, we were presented with a patient who was severely affected with probable TCDD poisoning. Without an established specific treatment protocol for such a severe and painful disease, the two possible treatment strategies were to continuously monitor the poison, its form, distribution, and elimination, and to search for medical molecular-based solutions for the organs that were affected by the toxin. We report here the first strategy because the specific wish of the patient was that his case contributed to increasing scientific knowledge about TCDD toxicity.

Methods

We obtained written approval from the patient to release peer-reviewed scientific information about his case. In early January, 2005, we identified TCDD (108 000 pg/g lipid weight) in our 50-year-old patient's blood serum, drawn under controlled conditions at the Geneva University Hospital, Switzerland (table 1), which was more than 50 000 times the average levels of TCDD in the general population.[17] Similar levels were identified by an independent laboratory in a sample taken from the same patient in mid-December, 2004.[18]

The patient's faeces were first lyophilised after homogenisation, whereas solid tissue samples (adipose tissue and skin) were frozen in liquid nitrogen and then homogenised by grinding with a pestle and mortar. Blood, urine, and sweat were frozen. We extracted and cleaned all the samples with different organic solvents, and then analysed the solvent extracts separately for the presence of TCDD metabolites. We mixed the samples with 17 standard ^{13}C12-labelled 2,3,7,8-chlorosubstituted PCDDs and PCDFs before we measured concentrations of TCDD and its metabolites. Because only the levels of TCDD, and not the other 16 chlorinated congeners, were higher in the patient than the levels in the general population, we only measured concentrations of TCDD in subsequent analyses. We used gas chromatography and high-resolution mass spectrometry to identify and quantify TCDD and its possible metabolites in the samples. The presence of possible TCDD metabolites was investigated on the basis of those predicted by Van den Berg and colleagues,[3] and identified by the analysis of the four most abundant signals of the chlorine isotope patterns within the expected molecular ion clusters in the selected ion monitoring mode during gas chromatography and high-resolution mass spectrometry. Because reference standards for hydroxylated dibenzo-p-dioxins were not commercially available, we used ^{13}C12-labelled 3,3′,4,5′-tetrachloro-4′-hydroxybiphenyl (Cambridge Isotope Laboratories, Andover, MA, USA) for quantification of the possible metabolites. This compound was chosen because it was structurally similar to hydroxylated TCDD metabolites and had a similar fragmentation pattern during electron ionisation mass spectroscopy.[19] Trichloromethoxydibenzo-

Figure 2: Photographs of Victor Yushchenko before poisoning (A), and 3 months (B) and 3·5 years (C) after poisoning with 2,3,7,8-tetrachlorodibenzo-p-dioxin

	Serum		Subcutaneous fat		Faeces		Skin biopsies		Materials extracted from skin		Sweat	Urine
	LW*	WW	LW	WW	LW	WW	LW	WW	LW	WW	WW	WW
4·01	108 000	860	89 000	38 000	..	..	7400	1200	116 000	1400	..	5
5·19	68 500	470	92 000	66 000	..	..	..	..	..	..	..	..
5·85	110 000	720	..	..	..	..	..	..	..	..	..	..
6·64	81 500	700	..	..	..	..	..	..	67 000	2400	..	..
7·40	75 750	680	..	..	..	..	..	..	..	..	..	..
9·40	73 500	550	..	..	..	..	..	..	..	..	..	..
9·80	69 250	600	68 000	50 000	..	..	21 000	1600	..	..	..	..
10·98	58 000	480	..	..	28 000	990	29 000	1150	58 000	3500	..	..
12·66	57 000	400	..	..	..	..	..	..	..	..	..	..
14·37	57 750	390	47 000	24 000	..	..	..	..	14 000	580	..	..
15·52	50 000	390	..	..	..	..	51 000	9200	..	..	..	..
17·36	46 750	400	48 000	39 000	..	..	..	2900	7900	520	..	..
18·67	47 000	320	..	..	..	..	..	..	14 000	240	..	..
20·74	47 000	310	..	..	..	..	..	..	18 000	270	..	..
22·42	..	..	..	..	9900	160	..	..	..	..	..	..
22·98	34 500	260	39 000	29 000	11 000	1100	39 000	930	23 000	1200	..	..
23·01	..	..	..	..	5900	200	..	..	..	..	4	..
23·08	..	..	..	..	10 000	820	..	..	..	..	..	0·05
28·47	31 250	260	30 500	19 000	13 000	135	..	..	..	..	..	0·03
30·77	28 000	230	..	..	..	..	..	..	..	..	..	..
34·12	25 750	180	28 750	23 000	..	..	..	..	..	..	..	..
39·25	20 500	160	23 000	11 000	3800	460	..	160	19 000	330	..	..

*Mean values from gravimetric and blood analyses.

Table 1: **2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) concentrations (pg/g lipid weight [LW] or pg/g wet tissue weight [WW]) as a function of time (months) after the day of poisoning in samples analysed**

p-dioxin and tetrachloromethoxydibenzo-p-dioxin, used as reference compounds for TCDD metabolites, were prepared in situ, whereas five of six possible monohydroxytetrachlorodibenzo-p-dioxins were provided. The lipid content of all samples was measured gravimetrically after evaporation of the solvent.

Equation 1 was used to calculate the decay of TCDD

$$\text{TCDD concentration (t)} = 110\,000\ (\text{pg/g serum lipids}) \cdot e^{-0{\cdot}045 \cdot t}$$

In this equation, t was time expressed in months, TCDD concentration was expressed as pg/g lipid weight, e was 2·71828, and t0 was the date of poisoning. The half-life was calculated from equation 1—ie, ln(2)/0·045=15·4 months. We used a period of 1 year, starting 11 months after the poisoning to try to correlate the TCDD decay curves with the TCDD eliminated or recovered from different routes. The concentrations of TCDD in the lipids at the start and end of this period were calculated with equation 1. The patient's body fat was calculated with a formula reported by Gallagher and colleagues,[20] and corroborated with CT imaging analysis.

The amount of TCDD eliminated in faeces, urine, and sweat during the 12 months of analysis was calculated with equation 2 as follows

$$\Delta m = \int_0^{12} m \cdot e^{-K \cdot t} \cdot dt = \frac{m}{K}(1 - e^{-12K})$$

In this equation, dt was the differential of time, m was the estimated mass of TCDD eliminated in the faeces, urine, or sweat during the first month—ie, the product of TCDD concentration at t0 and the amount eliminated in 1 month, and K was the decay (or rate) constant.

The frequent surgical interventions during which many skin biopsies were taken and cutaneous lesions were removed also represented routes of TCDD elimination. Equation 3 was used for the calculation of

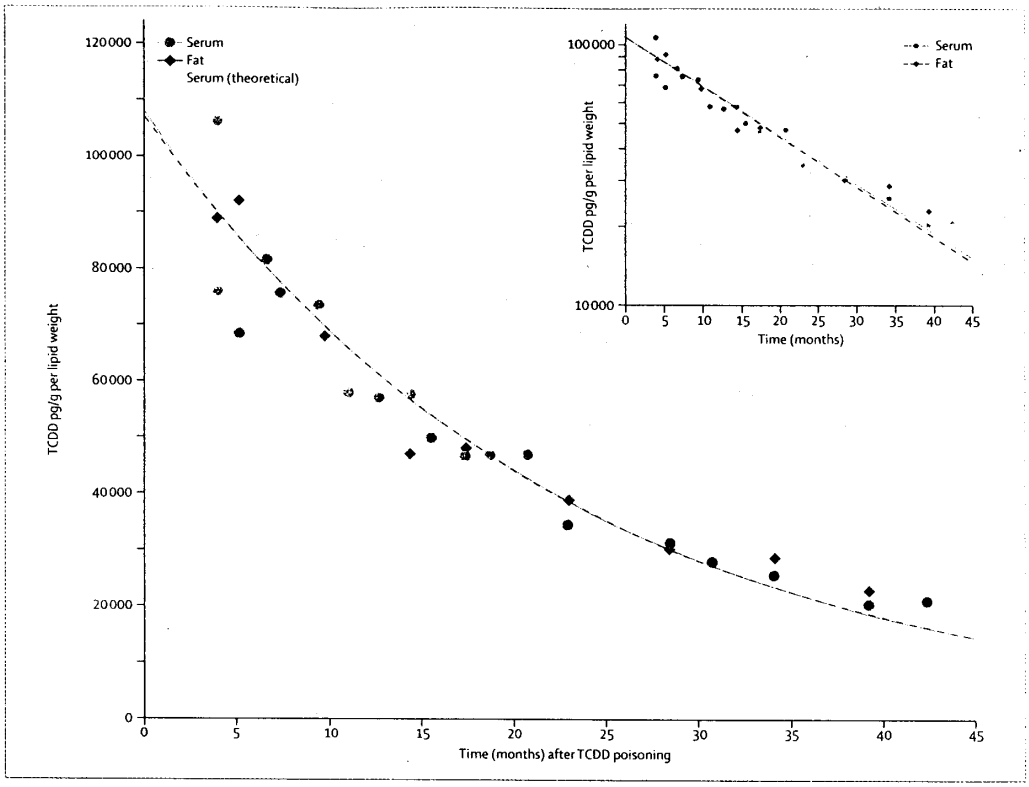


Figure 3: **Elimination decay curves of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in serum lipids and subcutaneous fat**
A theoretical decay curve is shown with values reported in individuals exposed to high levels of TCDD (y-axis is log scale in inset graph).[16] Green dotted line represents the expected decay curve for individuals not at risk of TCDD exposure. The fitted equation of TCDD decay in blood serum was (108 000±6000) (ppt) $e^{(-0.0449 \pm 0.0053)t}$, and in fat was (107 000±5000) $e^{(-0.0443 \pm 0.0035)t}$.

the two-phase kinetics (subscripts refer to the phases) shown by these skin biopsies and removed lesions

$$\Delta m = \int_{11}^{23} [(m_1 \cdot e^{-K_1 \cdot t}) + (m_2 \cdot e^{-K_2 \cdot t})] \cdot dt = \frac{m_1}{K_1}(e^{-11K_1} - e^{-23K_1}) + \frac{m_2}{K_2}(e^{-11K_2} - e^{-23K_2})$$

The two-phase kinetics can be explained by an accumulation phase from the blood and fat in the first stage, which lasted several months, followed by an exponential decay. About 200 samples of materials extracted from the skin were removed for analysis each month during 12 months. These materials contained skin (epidermis and dermis), blood, fat, and dermal cysts (webappendix).

See Online for webappendix

Role of the funding source

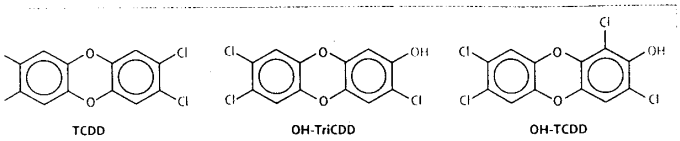
The sponsor of the study had no role in study design, data gathering, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We measured the concentrations of TCDD in serum lipids and subcutaneous fat samples from Victor Yushchenko over 3 years. The decay curves of TCDD in serum lipids and subcutaneous fat samples, calculated with first-order kinetics (figure 3), were similar, providing confirmation that TCDD was in equilibrium between serum lipids and subcutaneous fat.

The concentrations of TCDD in the lipids at the start and at end of the period of analysis were 66 000 pg/g and 38 000 pg/g, respectively. TCDD burden at the start and at end of the period of analysis was 990 µg and 740 µg, respectively. The amount of TCDD eliminated from the body during this time was 250 µg.

Table 2 shows the fitted values (ie, to the analytic curves) for the estimated TCDD eliminated per month (m_1) and the decay constant (K_1). The amount of TCDD eliminated, using equations 2 and 3, in the faeces, urine, and sweat, and during the surgical procedures that were done during 1 year was about 150 µg, representing 60% of total (250 µg) eliminated by the



ure 4: Structure of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and its metabolites 2,3,7-trichloro-8-troxydibenzo-p-dioxin (OH-TriCDD) and 1,3,7,8-tetrachloro-2-hydroxydibenzo-p-dioxin (OH-TCDD)

that are specifically designed to assess these effects are needed.

The TCDD concentrations in serum lipids and in subcutaneous fat recorded in this patient were similar during the 3 years of decay. Our set of nine analyses of TCDD and its metabolites in nine samples of adipose tissue and concurrent levels in blood, for the same person at various time points, confirm the findings of previous studies—ie, the existence of an equilibrium between these two compartments in man.[27] Our results in addition to the findings of previous studies should have an important implication for the design of strategies to monitor the exposure of individuals to toxins with new metabolomic approaches.

The measurement of TCDD concentrations in blood, adipose tissue, faeces, urine, and skin with time indicates that the samples extracted from TCDD-induced skin lesions contained large amounts of TCDD (table 2). These findings suggest that the toxin-induced skin lesions might represent a compartment that was not previously reported. Nonetheless, we have established that the main route of TCDD elimination in our patient was in the faeces, as previously reported in rodents.[28–30]

To understand the discrepancy between the amounts of TCDD eliminated in the faeces with time and its faster than predicted elimination half-life, we used a technical approach to search for TCDD metabolites in the samples from our patient. We identified two of five possible hydroxylated metabolites that were predicted by Van den Berg and colleagues.[5] These metabolites accounted for less than 40% of total TCDD eliminated in a man exposed to high levels of TCDD. The highest levels of metabolites were detected in faeces, whereas only traces were found in the blood serum. The metabolite to TCDD ratio was 50-fold lower in the blood serum than in faeces. These findings indicate that these metabolites were unlikely to have been ingested with TCDD, and that TCDD is slowly metabolised, probably by the liver and skin. In the skin, the genes encoding the CYP1A1 and CYP1A2 hydroxylases were highly induced, as assessed with quantitative PCR, whereas the enzymatic activity of CYP1A2 was substantially induced in the blood serum with the Cooperstown (5+1) cocktail method[31] (data not shown). High concentrations of TCDD might be needed to activate these phase I enzymes,[8,15,32] and therefore might explain why the TCDD half-life depends on the degree of exposure to TCDD. Although their chemical structure had not been elucidated, the possible occurrence of TCDD metabolites has previously been shown in rats,[33] dogs,[19] and human beings[34] with radiolabelled TCDD. Hydroxylated metabolites of the brominated analogue of TCDD—ie, 2,3,7,8-tetrabromodibenzo-p-dioxin—have been identified in rat bile.[35]

Although not done previously, levels of TCDD and its metabolites in tissue, faeces, and body fluids should be monitored in a patient with severe dioxin poisoning because they are indicators of what the follow-up period and treatment strategy should be. The poisoning of Victor Yushchenko with TCDD has changed from a story reported in the news to a medical model. This model of TCDD poisoning indicates that methods need to be designed for the routine analysis of TCDD metabolites in human beings, and the main aims of research into TCDD poisoning in the metabolomic era should be the analysis of factors that are involved in the metabolism of this toxin.[36]

Contributors

OS contributed to the study design, literature search, data analysis and interpretation, writing the report, and drawing figures. JHS contributed to the study design, literature search, patient care, data analysis and interpretation, and writing the report. MZ and PS contributed to literature search, data analysis and interpretation, and writing the report. OG, RF, RV, and VK participated in gathering samples and patient care. All authors have seen the final version of this report.

Conflicts of interest

We declare that we have no conflicts of interest.

Acknowledgments

This study was supported by the University of Geneva, Dermatology Foundation, and Swiss Centre for Applied Human Toxicology, Geneva, Switzerland; the authors had the freedom to use the funds for their research on dioxin poisoning. We thank the patient for his ability to cope with this disease and his willingness to contribute to increasing scientific knowledge about TCDD toxicity; Norbert Heeb, Andreas C Gerecke, and Hans-Rudolf Buser for helpful discussions; Heldur Hakk and Janice K Huwe from the US Department of Agriculture, Fargo, ND, USA, for providing reference compounds of hydroxylated TCDD; and Richard W James for his careful reading of the report and helpful comments.

References

1 Holt E. Doctor sues clinic over Yushchenko poisoning claims. *Lancet* 2005; 365: 1375.

2 Rosenthal E. Liberal leader from Ukraine was poisoned. *New York Times*, Dec 12, 2004. http://www.nytimes.com/2004/12/12/international/europe/12ukraine.html?_r=1&scp=1&sq=Liberal leader from Ukraine was poisoned Dec 12 2004&st=cs (accessed July 23, 2009).

3 BBC News. Tests confirm Yushchenko poison. June 2, 2006. http://news.bbc.co.uk/2/hi/europe/5040378.stm (accessed July 7, 2009).

4 Poland A, Knutson JC. 2,3,7,8-tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Annu Rev Pharmacol Toxicol* 1982; 22: 517–54.

5 Van den Berg M, De Jongh J, Poiger H, Olson JR. The toxicokinetics and metabolism of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) and their relevance for toxicity. *Crit Rev Toxicol* 1994; 24: 1–74.

6 Denison MS, Nagy SR. Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. *Annu Rev Pharmacol Toxicol* 2003; 43: 309–34.

7 Mimura J, Fujii-Kuriyama Y. Functional role of AhR in the expression of toxic effects by TCDD. *Biochim Biophys Acta* 2003; 1619: 263–68.

8 Rifkind AB. CYP1A in TCDD toxicity and in physiology-with particular reference to CYP dependent arachidonic acid metabolism and other endogenous substrates. *Drug Metab Rev* 2006; 38: 291–335.

9 Kohle C, Bock KW. Coordinate regulation of phase I and II xenobiotic metabolisms by the Ah receptor and Nrf2. *Biochem Pharmacol* 2007; 73: 1853–62.

10 Sulistyaningdyah WT, Ogawa J, Li QS, et al. Metabolism of polychlorinated dibenzo-p-dioxins by cytochrome P450 BM-3 and its mutant. *Biotechnol Lett* 2004; 26: 1857–60.

11 McKinley MK, Kedderis LB, Birnbaum LS. The effect of pretreatment on the biliary excretion of 2,3,7,8-tetrachlorodibenzo-p-dioxin, 2,3,7,8-tetrachlorodibenzofuran, and 3,3ê,4,4ê-tetrachlorobiphenyl in the rat. *Fundam Appl Toxicol* 1993; 21: 425–32.

12 Olson JR, McGarrigle BP, Gigliotti PJ, Kumar S, McReynolds JH. Hepatic uptake and metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzofuran. *Fundam Appl Toxicol* 1994; 22: 631–40.

13 Kedderis LB, Andersen ME, Birnbaum LS. Effect of dose, time, and pretreatment on the biliary excretion and tissue distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. *Fundam Appl Toxicol* 1993; 21: 405–11.

14 Landi MT, Bertazzi PA, Baccarelli A, et al. TCDD-mediated alterations in the AhR-dependent pathway in Seveso, Italy, 20 years after the accident. *Carcinogenesis* 2003; 24: 673–80.

15 Okino ST, Quattrochi LC, Pookot D, Iwahashi M, Dahiya R. A dioxin-responsive enhancer 3ê of the human CYP1A2 gene. *Mol Pharmacol* 2007; 72: 1457–65.

16 Aylward LL, Brunet RC, Carrier G, et al. Concentration-dependent TCDD elimination kinetics in humans: toxicokinetic modeling for moderately to highly exposed adults from Seveso, Italy, and Vienna, Austria, and impact on dose estimates for the NIOSH cohort. *J Expo Anal Environ Epidemiol* 2005; 15: 51–65.

17 Wittsiepe J, Furst P, Schrey P, et al. PCDD/F and dioxin-like PCB in human blood and milk from German mothers. *Chemosphere* 2007; 67: S286–94.

18 Brouwer A, Botschuiver S, Veerhoek D, et al. Observation of an extremely high dioxin level in a human serum sample from Ukraine by DR CALUX which was confirmed to be 2,3,7,8-tetrachlorodibenzo-p-dioxin by GC-HRMS. *Organohalogen Compounds* 2005; 67: 1705–08.

19 Poiger H, Buser HR, Weber H, Zweifel U, Schlatter C. Structure elucidation of mammalian TCDD-metabolites. *Experientia* 1982; 38: 484–86.

20 Gallagher D, Heymsfield SB, Heo M, Jebb SA, Murgatroyd PR, Sakamoto Y. Healthy percentage body fat ranges: an approach for developing guidelines based on body mass index. *Am J Clin Nutr* 2000; 72: 694–701.

21 Geusau A, Tschachler E, Meixner M, et al. Olestra increases faecal excretion of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Lancet* 1999; 354: 1266–67.

22 Steele EJ, Bellet AJ, McCullagh PJ, Selinger B. Reappraisal of the findings on Agent Orange by the Australian Royal Commission. *Toxicol Lett* 1990; 51: 261–68.

23 Michalek JE, Pirkle JL, Needham LL, et al. Pharmacokinetics of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Seveso adults and veterans of operation Ranch Hand. *J Expo Anal Environ Epidemiol* 2002; 12: 44–53.

24 Kerger BD, Leung HW, Scott P Jr, et al. Age- and concentration-dependent elimination half-life of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Seveso children. *Environ Health Perspect* 2006; 114: 1596–602.

25 Yoshimura T. Yusho in Japan. *Ind Health* 2003; 41: 139–48.

26 Geusau A, Schmaldienst S, Derfler K, Papke O, Abraham K. Severe 2,3,7,8-tetrachlorodibenzo- p-dioxin (TCDD) intoxication: kinetics and trials to enhance elimination in two patients. *Arch Toxicol* 2002; 76: 316–25.

27 Patterson DG Jr, Needham LL, Pirkle JL, et al. Correlation between serum and adipose tissue levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin in 50 persons from Missouri. *Arch Environ Contam Toxicol* 1988; 17: 139–43.

28 Pohjanvirta R, Vartiainen T, Uusi-Rauva A, Monkkonen J, Tuomisto J. Tissue distribution, metabolism, and excretion of 14C-TCDD in a TCDD-susceptible and a TCDD-resistant rat strain. *Pharmacol Toxicol* 1990; 66: 93–100.

29 Jackson JA, Birnbaum LS, Diliberto JJ. Effects of age, sex, and pharmacologic agents on the biliary elimination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in F344 rats. *Drug Metab Dispos* 1998; 26: 714–19.

30 Abraham K, Wiesmuller T, Brunner H, Krowke R, Hagenmaier H, Neubert D. Elimination of various polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs and PCDFs) in rat faeces. *Arch Toxicol* 1989; 63: 75–78.

31 Chainuvati S, Nafziger AN, Leeder JS, et al. Combined phenotypic assessment of cytochrome p450 1A2, 2C9, 2C19, 2D6, and 3A, N-acetyltransferase-2, and xanthine oxidase activities with the "Cooperstown 5+1 cocktail". *Clin Pharmacol Ther* 2003; 74: 437–47.

32 Walker NJ, Crofts FG, Li Y, et al. Induction and localization of cytochrome P450 1B1 (CYP1B1) protein in the livers of TCDD-treated rats: detection using polyclonal antibodies raised to histidine-tagged fusion proteins produced and purified from bacteria. *Carcinogenesis* 1998; 19: 395–402.

33 Poiger H, Schlatter C. Biological degradation of TCDD in rats. *Nature* 1979; 281: 706–07.

34 Wendling JM, Orth RG, Poiger H. Determination of [3H]-2,3,7,8-tetrachlorodibenzo-p-dioxin in human feces to ascertain its relative metabolism in man. *Anal Chem* 1990; 62: 796–800.

35 De Jongh J, Buser HR, Poiger H. The metabolism of 2,3,7,8-tetrabromodibenzodioxin in the rat. *Xenobiotica* 1993; 23: 19–26.

36 Nicholson JK, Lindon JC. Systems biology: metabonomics. *Nature* 2008; 455: 1054–56.