ABSORPTION OF HUMAN DIETARY INTAKE OF DIOXIN-LIKE COMPOUNDS (DLCs)

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Introduction

While many surveys of concentrations of dioxin-like chemicals (DLCs) – *viz* PCDD/Fs and PCBs - in human foodstuffs have been carried out, understanding of their uptake and elimination in humans is limited. Several studies[1-3] have reported that absorption of PCDD/Fs and PCBs in breast-fed infants exceeds 90% of the intake. In contrast, for older age groups, the evidence is that absorption is much less efficient, with excretion exceeding intake for some congeners[4-6] In summary, these studies suggest that – in addition to non-absorption of intake - DLCs in human faeces arise as a result of one or more of the following:

- endogenous excretion of elevated past intake
- *in vivo* biosynthesis
- the existence of other significant non-dietary intake pathways for DLCs

It has been suggested that absorption of DLCs across the GI-tract occurs *via* passive diffusion; in essence, the efficiency of absorption of dietary DLCs will depend on the concentration gradient existing between an individual's diet and serum – *i.e.* efficient absorption will occur when concentrations in diet are higher than those in serum, and *vice versa*. The objectives of this study were therefore:

- To study the absorption of the ingested human dietary intake of the following DLCs: the seventeen 2,3,7,8-chlorinated PCDD/Fs, and PCB congeners: 18, 28, 31, 37, 47, 49, 51, 52, 77, 81, 99, 101, 105, 114, 118, 123, 126, 128, 138, 153, 156, 157, 167, 169, 180, and 189. In particular, we wished to distinguish between that fraction of faecally-excreted material due to non-absorbed intake and that due to the sum of endogenous excretion and *in vivo* biosynthesis.
- To evaluate the validity of the hypothesis that human absorption of DLCs is a passive diffusion process.

Consequently, for each individual subject in this study, two separate experiments were held. In the first, the subjects' "normal" omnivorous diets were administered, whilst in the second, the effect of a "low-DLC" diet (vegan – *i.e.* no animal products) was studied. The intention of the "low-DLC" diet was to minimise the excretion of DLCs excreted due to non-absorption of dietary intake, to the extent that DLCs excreted during this second trial would essentially comprise the sum of endogenous excretion and *in vivo* biosynthesis alone. As a result, it was hypothesised that subtraction of DLCs excreted during the "low-DLC" diet experiments from that excreted during the "normal-diet" experiments would provide an indirect (or "corrected") estimate of DLCs excreted as a result of non-absorption of dietary intake. Furthermore, if GI-absorption of DLCs

occurs *via* passive diffusion, then one would anticipate that younger individuals (who have lower body burdens) would absorb DLCs more efficiently than older individuals. To test this hypothesis, we studied 2 groups of 7 volunteers: one aged 23.9±4.5 years and the other aged 49.4±5.2 years. To minimise variations in absorption behaviour due to sex and body mass index (BMI), all subjects were male, with BMI values 23.1±2.2 (younger group) and 23.2±2.5 (older group).

Materials and Methods

Study Design and Sample Collection

A "real-time" mass balance approach was employed in this study. For each individual subject, two separate experiments were held. In the first, the subjects' normal diet (ascertained by questionnaire) was administered, whilst in the second, the effect of a vegan diet was studied. Subjects' meals were taken in the metabolic unit of the Department of Nutrition and Dietetics of King's College, London. Where food was consumed outside of the metabolic unit, then subjects kept detailed notes and provided duplicates of what was consumed. Individual duplicate diet samples of all food and drink were collected daily in pre-cleaned glass jars and aluminium boxes, for the full duration of each trial. To match dietary intake with faecal excretion, $BaSO_4$-coated PVC pellets were used as a biological tracer, with pellets eaten at breakfast on day 1 and with the first meal on day 8. Faeces were collected daily in aluminium foil lined plastic sealable containers over a 10 day period from day 1. The first faecal specimen containing the first marker was included in the collection with all subsequently passed specimens until the first appearance of the second marker. Specimens containing the second marker and all subsequently passed specimens were excluded. At the end of each trial, the whole of the "matched" 7-day diet and faeces samples for each subject were weighed, thawed and homogenised. Appropriate quantities of each homogenate were immediately freeze-dried and stored at –18°C until analysis.

Analytical Methods

PCDD/Fs and PCB #s 77, 126, and 169 These were determined using GC high resolution MS using an Autospec Ultima instrument. The seventeen 2,3,7,8-chlorinated PCDD/Fs were analysed at Scientific Analysis Laboratories Ltd (SAL), SAL use an in-house analytical method based on USEPA Method 1613. Their method for the determination of PCB #s 77, 126, and 169 was based on that of Harrad *et al* [7]

PCBs other than #s 77, 126, and 169 The method used was based on that reported previously[8], with some modification of the purification procedures as necessary for optimum performance for duplicate diet and faeces samples. Sample extracts were analysed using a Fisons' MD800 GC/MS operated at unit mass resolution.

Calculation of Absorption of Human Dietary Intake of DLCs

Concentrations of individual DLCs in diet and faeces samples were used to calculate the net absorption of dietary intake for a given subject during a given week's trial. The first approach is that used in previous studies, and referred to here as the "simple" approach, whereby net absorption is calculated thus:

$$\% \text{ net absorption of dietary intake} = (1 - E / I) \times 100 \qquad (1)$$

where E = excreted mass and I = ingested mass

However, this "simple" approach can – as discussed earlier – lead to underestimates of absorption,

due to endogenous excretion of elevated past intake. In an attempt to "correct" for such endogenous excretion, we considered excretion and intake data from both weeks' trials for each subject, and calculated absorption *via* what is termed here as the "corrected" approach:

$$\% \text{ absorption of dietary intake} = [1-((E_{ND} - E_{LDD})/(I_{ND} - I_{LDD}))] \times 100 \quad (2)$$

where E_{ND} = excreted mass during normal diet trial, E_{LDD} = excreted mass during the low DLC diet trial, I_{ND} = ingested mass during normal diet trial; and I_{LDD} = ingested mass during low DLC diet trial

Results and Discussion

Dietary intakes for this study are discussed in detail elsewhere[9]. A summary of the results for the entire study is as follows:

For both subject age groups, despite DLC intakes during the vegan diet trials being generally significantly lower than those during the normal diet trials, DLC excretion during the vegan diet trials was generally either comparable to or exceeded that observed during the normal diet trials. This suggests that the majority of DLCs detected in human faeces occur as a result of endogenous excretion and/or *in vivo* biosynthesis.

For the younger subjects, the "corrected" approach to estimating DLC absorption efficiency revealed absorption of most DLCs to be greater than apparent *via* a simple mass balance comparison of excreted and ingested DLC masses. To illustrate, for OCDD mean absorption *via* the "corrected" approach was 67%, compared with 27% and -116% for normal and vegan diet mass balances respectively.

The "corrected" approach requires endogenous and biosynthetic excretion to occur at a broadly constant rate in both trials – or to be relatively low compared to intake – as where this occurs, even large percentage variations in excretion exert little influence on the "corrected" approach estimates. In contrast to the younger group, excreted masses were either close to or exceeded dietary intake in both trials for the older group, hence inter-trial variability in endogenous and/or biosynthetic excretion exerted a significant confounding influence on the estimates provided by the "corrected" approach, which were consequently deemed unreliable.

We conducted statistical comparison of net absorption/excretion data obtained for individual DLCs for both age groups during: (a) the normal diet; and (b) the vegan diet trials. To do so, we first evaluated the data using the Kolmogorov-Smirnov test to evaluate whether the distribution of absorption data for the 2 age groups was significantly different. If it was, then the non-parametric Mann Whitney *U*-test was used to test for significant differences in net absorption behaviour between the 2 age groups; otherwise the parametric t-test was utilised.

Comparison of data for the 2 age groups from the normal diet trials revealed absorption efficiency to be significantly *lower* for the older group for the following 9 DLCs: 2,3,7,8-TCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, 1,2,3,7,8-PeCDF, PCB 47, 99, 118, 105, and 153 ($P<0.05$). No statistically significant differences ($p>0.05$) were detected for any of the other target DLCs.

Comparison of data for the 2 age groups from the vegan diet trials revealed absorption efficiency to be significantly *lower* for the older group for the following 12 DLCs: OCDF, PCB 31, 28, 52, 49, 47, 101, 99, 118, 105, 153 and 138 ($P<0.05$). Net absorption was significantly *greater* for the older group for 2 DLCs, viz: 1,2,3,4,6,7,8-HpCDD, and 1,2,3,4,6,7,8-HpCDF ($p<0.05$). However, this is attributable to the fact that dietary concentrations of these congeners were significantly higher ($p<0.05$ using the Mann Whitney *U*-test) for the older group. No statistically significant differences ($p>0.05$) were detected for any of the other target DLCs. This greater absorption of some DLCs by the younger group provides evidence that the passive diffusion model of DLC transfer across the GI-tract is a reasonable approximation of the complex processes involved.

In common with previous studies, net absorption/excretion behaviour of DLCs varied widely between subjects, even within age groups of all male subjects that were quite closely matched for BMI, and – as in the vegan diet trials – ingested similar dietary fat concentrations of DLCs. This supports a previous suggestion[5] that inter-subject variations in net absorption/excretion behaviour are primarily attributable to variations in DLC concentrations in serum. Despite this, in contrast to a previous report of a positive relationship between net PCB absorption and body fat index[6], this study found no evidence of a relationship between BMI and DLC absorption efficiency.

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References

1. Abraham, K., Knoll, A., Ende, M., Päpke, O., and Helge, H. (1996) Pediatr. Res., 40, 671-679.
2. Korner, W., Dawidowsky, N., and Hagenmaier, H. (1993) Chemosphere, 27, 157-162.
3. McLachlan, M. S. (1993) Toxicol. App. Pharmacol., 123, 68-72.
4. Schrey, P., Wittsiepe, J., Mackrodt, P., and Selenka, F. (1998) Chemosphere., 37, 1825-1831.
5. Schlummer, M., Moser, G. A. and McLachlan, M. S. (1998) Toxicol. App. Pharmacol., 152, 128-137.
6. Juan, C-Y., Thomas, G. O., Jones, K. C. (2000) Organohalogen Compounds, 48, 338-341.
7. Harrad, S. J., Sewart, A. S., Boumphrey, R., Duarte-Davidson, R., and Jones, K. C. (1992) Chemosphere, 24,, 1147-1154.
8. Ayris, S., Currado, G.M., Smith, D., and Harrad, S. (1997) Chemosphere, 35, 905-917.
9. Wang, Y., Sandaradura, S., Leeds, A., and Harrad, S. (2001) ibid.