In vitro metabolism of tetrabromobisphenol A by Human and rat sub-cellular liver fractions

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INTRODUCTION

Tetrabromobisphenol (TBBPA) accounts for a large part of the world production of brominated flame retardants. It is used in the manufacture of plastics, textiles electronic boards and upholstering foams. Little is known about the fate of this chemical in the environment, and even less about its biotransformations in human. It was previously shown in rat that the main metabolic routes of TBBPA were the conjugation of one or both phenolic moieties of the molecule, to glucuronic acid or sulphate[1]. Using ^{14}C-labeled TBBPA, we carried out *in vitro* metabolic studies using human microsomal and S9 fractions (microsomes + cytosol), with similar experiments being carried out in rat for comparison. The metabolism of TBBPA was followed by HPLC with on-line radioactivity detection.

MATERIALS AND METHODS

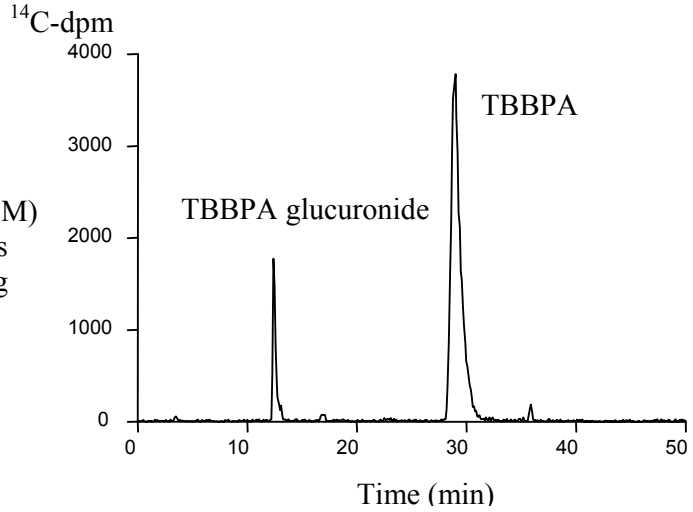
^{14}C-TBBPA was synthesized by bromination of ^{14}C-bisphenol A (Moravek Biochemicals, USA) following a method derived from Susãn *et al.*[2]. It was purified by thin layer chromatography and HPLC.
Rat sub-cellular liver fractions (microsomes and S9) were prepared from male and female Wistar rats (n=3). Pooled Human sub-cellular liver fractions from men and women were obtained from TEBU, France.
Incubations were carried out using 2,500 to 5,000 Bq ^{14}C-TBBPA, fortified with unlabeled TBBPA (Aldrich, France) to reach concentrations ranging from 20 to 500 µM. They were achieved at pH 7,4 with a duration of two hours. Control assays (no NADPH generating system) were carried out for all types of fractions.
Metabolic profiling was achieved by HPLC with on-line radioactivity detection, using methods derived from Zalko *et al.*[3]. Major metabolites were purified using C18 cartridges and HPLC separation, and their structure was investigated by mass spectrometry, using a LCQ Finnigan ion trap spectrometer (Thermo Quest, France) and electrospray as the ionization technique, in the negative mode.

RESULTS

^{14}C-TBBPA was obtained with a specific activity of 1264 Bq/µg. Its radio-purity, checked by HPLC, was 99.8 %.

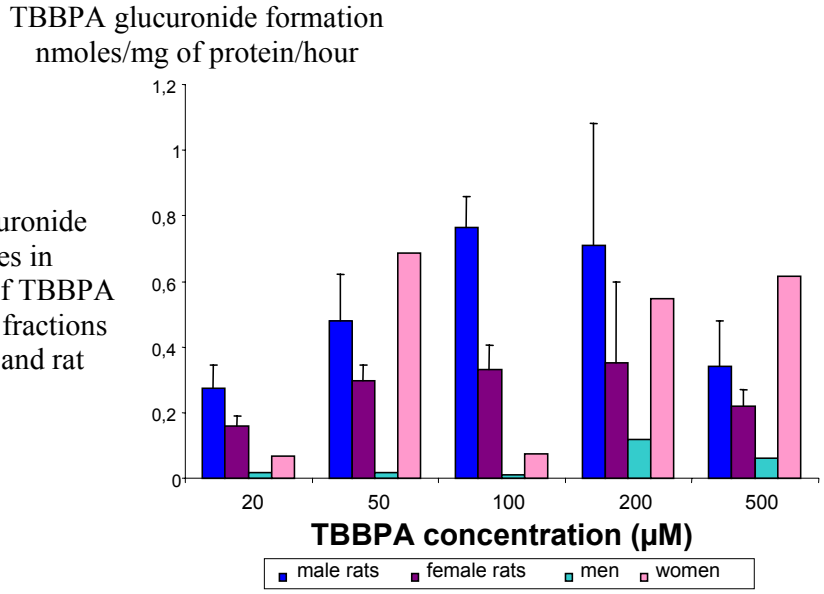
Following *in vitro* assays completion, radioactivity recovery from the incubation supernatants was greater than 90% for liver microsomal fractions, and close to 100% for liver S9 fractions, with no significant difference being observed between males and females, nor between rat and Human.
Liver S9 incubation supernatants analyzes, carried out without NADPH generating system, led to the formation of a single major metabolite with a retention time of 12.5 min (figure 1). This metabolite was shown to co-elute with the authentic standard TBBPA-glucuronide, previously synthesized at the laboratory.

Figure 1:
Radio-HPLC profile obtained after incubating TBBPA (20 µM) with male rat S9 liver fractions without an NADPH generating system.



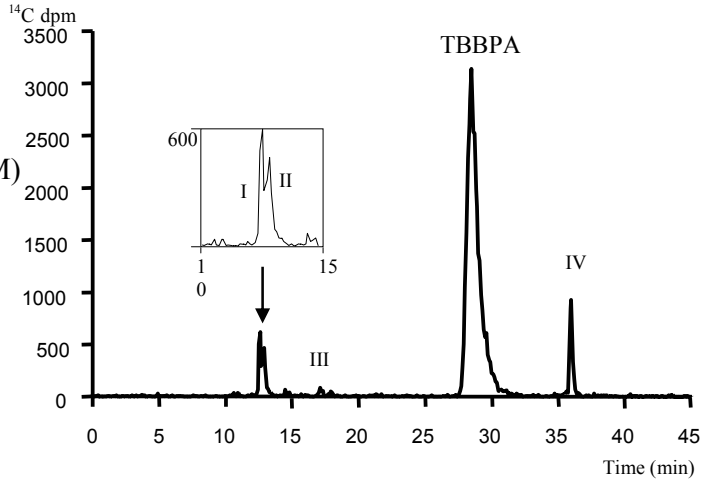
The structure of BPA glucuronide was confirmed by mass spectrometry. However, when TBBPA was incubated with sub-cellular fractions (either microsomes or S9) in the presence of a NADPH generating system, the formation of additional metabolites was observed. In these conditions, TBBPA-glucuronide formation rate (figure 2) reached a maximum of 0.8 nmoles/mg of protein/hour in male rats for incubations carried out with liver S9 fractions. TBBPA glucuronidation rates were found to be in the same range in men, but were much lower in female rat and women.

Figure 2:
TBBPA glucuronide formation rates in incubations of TBBPA with liver S9 fractions from Human and rat



Besides TBBPA glucuronide, several other metabolites were detected on radio-chromatograms (figure 3), including a metabolite exhibiting a lower polarity than unchanged TBBPA in our chromatographic system (metabolite IV). The formation of most of these compounds is P450-dependent, since they are not formed when the NADPH generating system is not added to incubation media. It is detailed for metabolite IV in figure 4. Metabolite III was identified as hydroxylated TBBPA. The structure of the other metabolites is still under investigation.

Figure 3:
Radio-HPLC profile obtained after incubating TBBPA (20 µM) with women S9 liver fractions



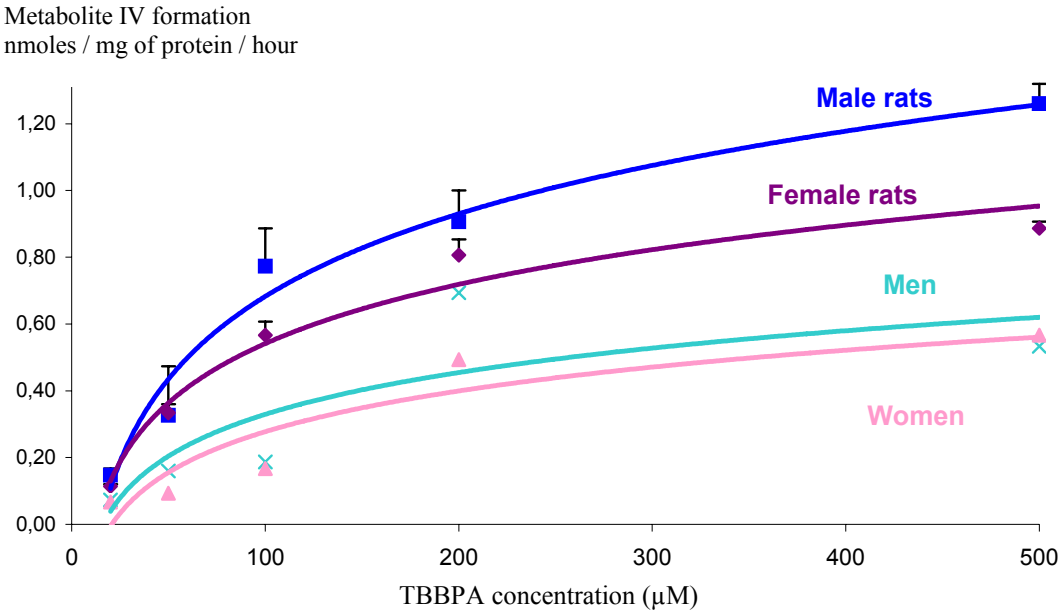


Figure 4: metabolite IV formation in incubations of TBBPA with liver S9 fractions from Human and rat

Radio-HPLC analyzes of the supernatants of incubations carried out with liver microsomes demonstrated the formation of two major metabolites, with similar retention times as metabolites II and IV, while no TBBPA glucuronide was produced, as expected under the experimental conditions that were setup for these incubations.

DISCUSSION AND CONCLUSIONS

The formation of TBBPA glucuronide occurs *in vitro* both when TBBPA is incubated with Human, or rat liver S9 fractions. However, other metabolic pathways are also involved in TBBPA metabolism, and the formation of some major *in vitro* metabolites of TBBPA, either with liver microsomes or with liver S9 fractions, appears to be P450 dependent. The structure of these metabolites is under investigation. Small amounts of hydroxy-TBBPA have been identified. Metabolites resulting from oxidative biotransformations of TBBPA are formed *in vitro*, and no major qualitative difference of metabolite profiling is observed between Human and rat, nor between males and females.

REFERENCES

(1) Hakk H, Larsen G, Bergman A, Orn U. Metabolism, excretion and distribution of the flame retardant tetrabromobisphenol-A in conventional and bile-duct cannulated rats. *Xenobiotica*. 30:881-890, 2000.

(2) Susãn AB, Ebert DA, Duncan WP. Synthesis of ^{14}C-labeled flame retardants. ^{14}C-labeled tetrabromophtalic anhydride and tetrabromobisphenol. *A. J. Label. Compound Radioph.* 16: 579-589, 1978.

(3) Zalko D, Soto AM, Dolo L, Dorio C, Rathahao E, Debrauwer L, Faure R, Cravedi JP. Biotransformations of bisphenol A in a mammalian model: answers and new questions raised by low-dose metabolic fate studies in pregnant CD1 mice. *Environ. Health Perspect.* 111:309-319, 2003.