Estrogenic Potency of Individual Nonylphenol Congeners Isolated from Technical Mixtures

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Abstract

Nonylphenol (NP) is an estrogenic compound found in various environmental media. The technical NP mixture consists of more than 20 isomers. Since toxicity, including estrogenic potency, and environmental persistency of each isomer can vary depending up on the physico-chemical properties, we isolated components of technical NP into six fractions using a two-dimensional gas chromatography technique, and tested for their ability to elicit estrogenic potential in an *in vitro* gene expression assay with MCF-7 cells. The technical NP mixture consists of both estrogenic and non-estrogenic isomers. Relatively a few isomers accounted for most of the estrogenicity of the technical mixture. Isomer-specific analysis of NP would be necessary for an accurate evaluation of estrogenic potency of nonylphenols found in the environment.

Introduction

Nonylphenol (NP) is extensively used for the manufacture of surfactants, antioxidants and other products. A major source of NP present in the environment is the degradation of nonylphenol polyethoxylates (NPE), which are nonionic surfactants used in detergent formulations. Concern about the environmental occurrence of NP and NPE has arisen because of their estrogenic effects to fish and mammal cells *in vitro* (1). NP is a mixture of more than 31 components (2). However, many researchers still measure NP as a single compound since there is no established analytical method for isomer-specific determination, a case similar to that for polychlorinated biphenyls (PCBs) until the early 1980s. The toxicity, including estrogenic potency, and the bioaccumulation potential of each NP isomer can vary depending on the structure and physico-chemical properties. In this report, we separated individual components of NP using two-dimensional capillary gas chromatograph equipped with a preparative fraction collector (2DGC-PFC). Each fraction was tested for its estrogenic potential using a recombinant cell line, MVLN (MCF-7) which are human breast cancer cells stably transfected with a luciferase reporter gene under control of the estrogen response element (ERE) of the *Xenopus* vitellogenin A2 gene.

Materials and Methods

Technical NP mixture was fractionated into six fractions using 2DGC-PFC (GERSTEL 8000 dual oven system with multi column switching and preparative faction collector). DB-5 (J & W Scientific, 0.53 mm i.d., 60m, 1.5μm film thickness) and HP-PONA (Hewlett-Packard, 0.2 mm

i.d., 50m, 0.5 μm film thickness) fused silica capillary columns were used as the first and the second columns, respectively. Tentative identification of each peak separated by the PONA column was done by using a high resolution gas chromatography – mass spectrometry (HRGC-LRMS; Hewlett-Packard 6890+ GC and 5973 MS) connected with HP-PONA column (1). Technical NP mixture (Kanto Chemicals Co.) was diluted to 3000 μg/ml in hexane and injected into the above systems using Gerstel large volume auto sampler. Five μl of standard was injected and repeated 100 times. Totally, more than one mg of NP was injected and fractionated using the solvent venting mode. Six fractions were collected using a glass capillary trap, which was kept chilled in water. The glass capillary trap was washed with hexane and the solvent was transformed into a glass vial. These solutions were applied for MCF-7 bioassay to determine estrogenic potency. Cultured MCF-7 cells were diluted to a concentration of approximately 75000 cells/mL and seeded into 96-well culture plates. Cells were incubated overnight and then dosed with fractionated NP isomer containing extracts. Three different concentrations of each fraction prepared by 10-fold dilution were tested. Further details of MCF-7 luciferase assay have been described elsewhere (3).

Results and Discussion

A total ion chromatogram (TIC) of NP isomers separated by the PONA column is shown in Figure 1. There were more than 20 prominent NP peaks that were found in technical NP standard. Various components of NP isomers that were present in each hexane fraction are also shown in Figure 1. Tentative identity of selected list of major NP peaks that were isolated into each fraction is given in Table 1. Fraction 1 contained two major components A and B along with several minor peaks, which elute relatively rapidly from the GC columns. Similarly, fraction 6 contained components M and N and several minor peaks, which have slower retention time. Other four fractions (fractions 2 to 5) contained major constituents of technical NP. Peak E was not separated from Peak D in the earlier report (2).

Results of estrogenic potency of the six fractions isolated from technical NP mixture are shown (Figure 2). The procedural blank is represented by the dotted line. In order to normalize the amount of estrogenicity contributed by each fraction, the estrogenic potency observed in sample extracts was subtracted from the procedural blank and divided by area of total ion chromatogram of each fraction (Figure 3). Estrogenic potency of the fraction 3 was the highest of all the six fractions examined. Fraction 3 had a TIC area of only 19% of the total NPs, but contained more than 46% of the total estrogenic potency of the technical NP mixture. Tentatively, this fraction contained (*p*) 3-methyl 3-phenolic octane and an unidentified component. It is remarkable that fractions 2 and 4 occupy more than 41% of TIC area but they represent only 7% of total estrogenicity of the technical NP mixture.

Although our results show only relative estimates of estrogenic potency of various NP components (because separation and identification of each component is not performed completely), it is worth to mention that 2DGC (or HRGC) - PFC is a useful technique to isolate individual components in a complex mixture. Additionally, it is clear that technical NP mixture consists of both estrogenic and non-estrogenic components. It would be interesting to examine bioaccumulation potential and environmental persistency of individual isomers which would help to provide information for making policy decisions. In other words, identification of non-estrogenic and non-persistent NP isomers in the technical mixture would indicate alternative, environmentally safe NP mixture, for use in various commercial applications.

Acknowledgements
We thank Yakuruto Company for supporting MCF-7 bioassay. We also thank Dr. Ryuichi Naganawa (NIRE) and Dr. Hideshige Takada (Tokyo University of Agriculture and Engineering) for their helpful comments on this study.

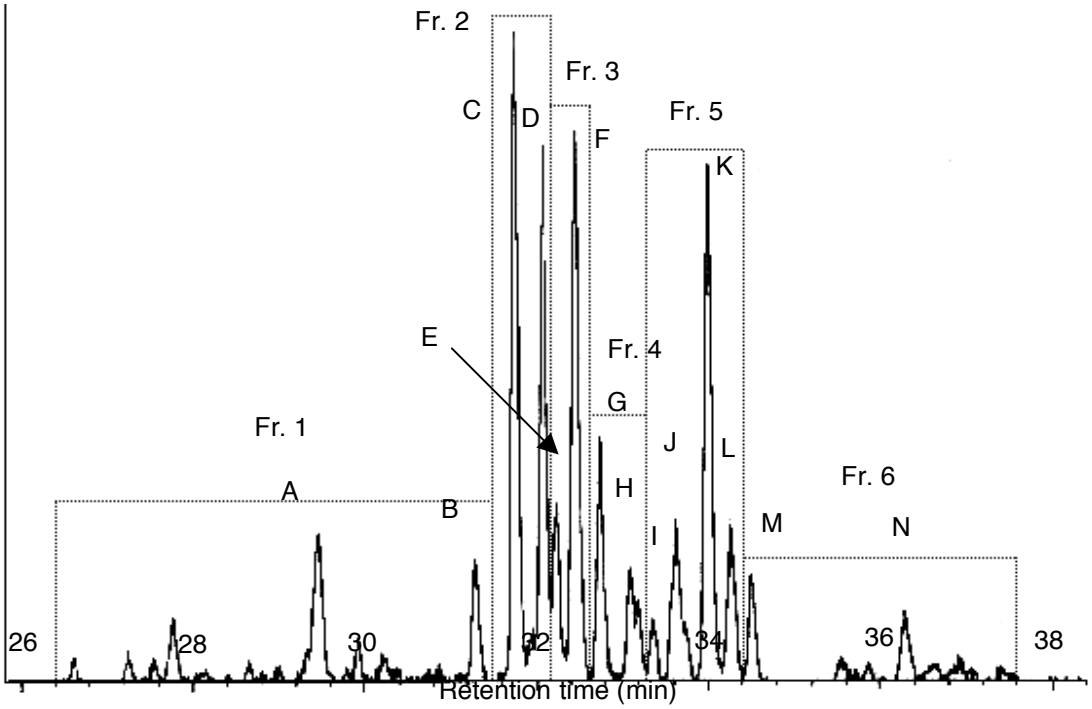

Fig. 1 Total ion chromatogram of NP mixture and various fractions collected by 2DGC-PFC.

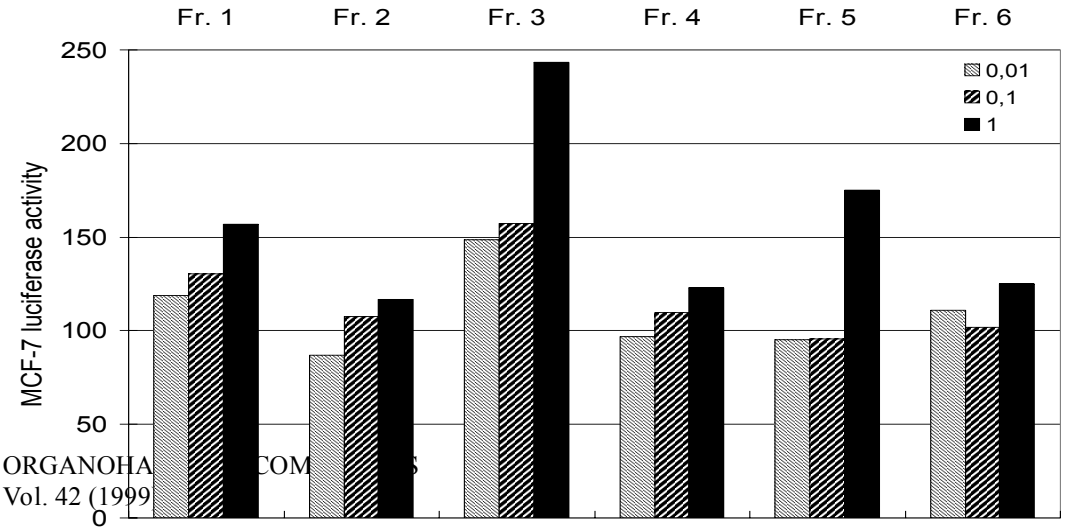

Fig. 2 MCF-7 luciferase activity of fractions of NP mixture.

Table 1 List of NP components fractionated by 2DGC-PFC and tentative identification by Bhatt et.al. (1992).

Fraction No.	Peak	Tentative identification	Peak	Tentative identification
Fraction 1	A	(*p*)2-phenolic 3,6-dimethyl heptane	B	(*p*)2-phenolic 4,5-dimethyl heptane
Fraction 2	C	(*p*)2-phenolic 3,4-dimethyl heptane	D	(*p*)3-methyl 2-phenolic octane
Fraction 3	E	unidentified	F	(*p*)3-methyl 3-phenolic octane
Fraction 4	G	(*p*)3-methyl 4-phenolic octane	H	(*p*)3-phenolic 5 -methyl octane
Fraction 5	I	(*p*)3-phenolic 4,5-dimethyl heptane ?	J	(*p*)2-phenolic 4,6-dimethyl heptane
	K	2-methyl 3-phenolic octane	L	(*p*)4-methyl 2-phenolic octane
Fraction 6	M	(*p*)3-phenolic 3,6-dimethyl heptane	N	(*o*)2-phenolic 4,6-dimethyl heptane ?

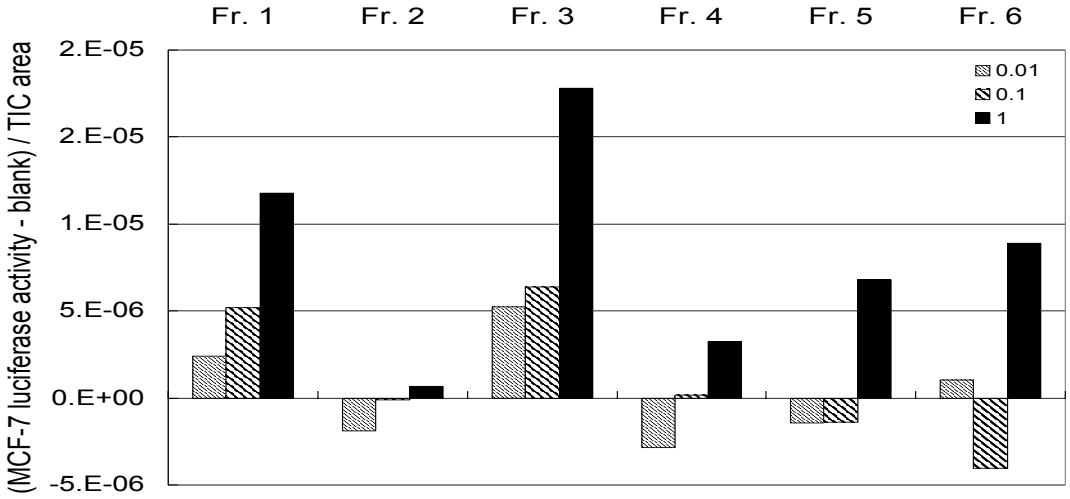

Fig. 3 Relative measure of MCF-7 luciferase activity (blank substracted, TIC area basis) in six fractions of technical NP mixture.

References

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