Preparation of molecularly imprinted polymers for heptachlor: An organochlorine pesticide

Abstract

Background: Molecular Imprinting technology is a promising technique for creating recognition elements for selected compounds and has been successfully applied for synthesis of Environmental pollutants such as pesticides. **Aim:** Synthetic binding polymers were prepared by non-covalent imprinting technique using bulk polymerization method for heptachlor, which is an organochlorine pesticide. **Materials and Methods:** Three functional monomers and 2 cross-linkers were used for molecular imprinting. The molecularly imprinted polymers (MIPs) obtained were subsequently characterized in detail with rebinding investigation using batch rebinding. The results indicate that the imprinting was successfully done for heptachlor using methacrylic acid (MAA) as functional monomer and ethylene glycol dimethacrylate (EDMA) as cross-linker. **Results:** The affinity order from rebinding results for the 3 polymers is heptachlor-co-MAA-co-EDMA > heptachlor-co-4-VP-co-DVB > heptachlor-co-Styrene-co-DVB. UV-visible spectrophotometric study for interaction of heptachlor with MAA in pre-polymerization mixture suggests the formation of H-bonding also contributing to the formation of good imprint. **Conclusion:** Molecular Imprinted polymers can be used as detection systems and as clean up materials for pesticides.

Key words:
Heptachlor, molecularly imprinted polymers, U.V. spectrophotometry

Introduction

Recent advances in the field of molecular imprinted polymers (MIPs) have created synthetic materials that can mimic the function of biological receptors with less stability constraints.[1] In molecular imprinting process, functional monomers are arranged around the template molecule and fixed in place by copolymerizing with cross-linking monomers. The result is a rigid matrix, which contains template molecules bound to the monomer by steric or chemical interactions. After removal of template, the polymer can be used as affinity material as it contains complementary site for template molecule. These polymers provide high sensitivity and selectivity while maintaining excellent thermal and mechanical stability.[2,3] Molecular imprinted polymers or synthetic antibodies have been used in a broad range of applications, mainly as affinity separation materials, antibody binding mimics, materials for molecular recognition, enzyme mimics, and as sensors.[2-9]

MIPS could not only help as detectors of environment toxicants but also as one step clean up sorbents/sieves for remediation of surface-borne residues in fruits and vegetables, water, and soil. Current sample clean-up methods like solid phase extraction methods are very fast and inexpensive but show lack of selectivity while methods based on immunoaffinity are very selective but expensive and are not suitable for harsh environments.[10,11] Molecular imprinted polymers have proved to be an excellent replacement for the above traditional extraction materials.[12,13]

MIPs have been synthesized for large and small molecules, herbicides including atrazine[14] and 2, 4, 5, trichlorophenoxyacetic acid,[15] environmental contaminants pentachlorophenol[16] and organophosphorus pesticides such as monocrotophos, paraoxon, dichlorovos.[17,18] However, except for a single report on DDT by sol-gel technique,[19] no reports on MIP synthesis are available for organochlorine pesticides. The reason

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could be the unavailability of functional groups suitable to imprint these molecules using covalent interactions or non-covalent interactions like hydrogen bonding between template and monomer molecules. The only forces that can be applied to the imprinting of these molecules are van der Waals forces, electrostatic interactions, dipole interactions, steric interactions, and π-π interactions which are much weaker, compared to electrostatic interactions but have been successfully applied for obtaining molecular imprints.

Scanty reports on imprinting of such poorly functionalized templates are available. Dickert *et al*.[20,21] reported molecular imprinting for polycyclic aromatic hydrocarbons (PAHs) naphthalene (2 rings) and perylene (4 rings). Imprinting was based on π-π interactions and van der Waals interaction. To provide an optimum interaction via π-π bonds, aromatic monomers like phloroglucinol and bisphenol - A were selected for imprinting of these poorly functionalized compounds. Lai *et al*.[22] developed a molecular imprint for benzopyrene; a 5 ring PAH based on hydrophobic and π-π interactions. A combination of different functional monomers and functional cross linkers was used to synthesize imprinted polymers.

The present study describes molecular imprinted polymers for heptachlor, an organochlorine pesticide. Heptachlor, a chlorinated dicyclopentadiene insecticide, is mainly used in the control of termites and soil insects. It slowly gets oxidized to heptachlor epoxide, which is more stable and toxic than heptachlor.[23] Although its use has been banned or severely restricted in many countries since the 1980s, it is still detected as a contaminant in soil, water, and food commodities.[24-29] This is mainly due to its persistence nature, but also suggests its illegal use in the recent past or present. It is known to be one of the most persistent insecticides,[30] and is also formed by the chemical modification of chlordane, which was used until 1988. Both heptachlor and its epoxide are persistent, highly toxic, can cause hyper excitability, tremors, convulsions, and paralysis[30-33] and are also known to have carcinogenic effects.[34]

For this study, 3 different monomers and 2 different cross-linkers were used to produce 3 different imprinted polymers for heptachlor. Conventional bulk polymerization method was used for the preparation. Selectivity and binding studies were performed. The polymer with the highest binding capacity for heptachlor was used as a sorbent for solid phase extraction of heptachlor from spiked soil samples.

Materials and Methods

Experimental section
Heptachlor [Figure 1] technical (purity 99%) was used as the template molecule. Methacrylic acid (MAA) (Sigma Aldrich) was purified using NaOH and distilled before use. Ethylene glycol dimethacrylate (EDMA), 4-vinyl pyridine (4-VP), divinyl-benzene (DVB), and styrene were purchased from Sigma and distilled prior to use to remove inhibitors. 2' 2-azobisisobutyronitrile (AIBN) was re-crystallized over ethanol before being used. All other chemicals were of analytical grade and used as supplied.

All spectrophotometric measurements were made with an ultraviolet visible recording spectrophotometer (UV-265, Shimadzu, Japan) with matched 1-cm quartz cells. In order to compare all spectrophotometric measurements and ensure reproducible experimental conditions, the UV-265 spectrophotometer was checked daily in wavelength accuracy and linearity. A constant temperature bath (Shruthi Electronics, Bangalore, India) was used for controlling temperature (60±0.1 °C). HPLC analysis was done on HPLC system with a Shimadzu LC-10AT pump and UV detector (Shimadzu, Columbia, MD, USA). HPLC column used was PARTISIL 5 ODS-3 WCS Analytical column 4.6 mm × 250 mm (Whatman International Ltd., Maidstone, England). Analysis was done using isocratic system with mobile phase methanol: Water (3:1 v/v) with UV detection at 220 nm. Flow rate was kept at 1 ml/min. Retention time for heptachlor was 21.5 minute.

Preparation of imprinted polymers for heptachlor
Template, functional monomer, and porogen were accurately weighed [Table 1] into 25 ml round-bottomed flask and were allowed to mix for 15 minutes. Three polymers were made with the following combinations: Heptachlor-co-MAA-co-EDMA (P1), heptachlor-co-4-VP-co-DVB (P2),
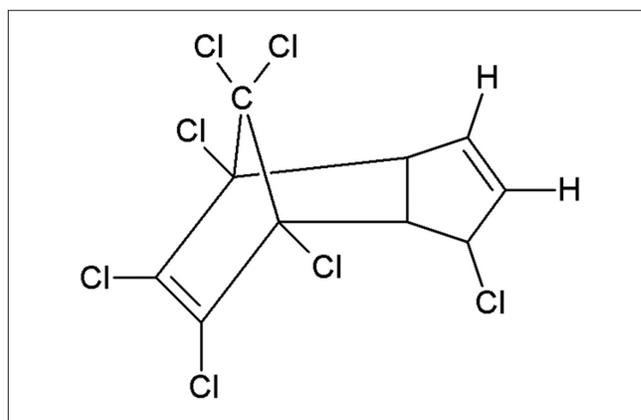
Figure 1: Chemical structure of heptachlor

Table 1: Selected monomers and cross-linkers combination for different polymers

Code	P1	P2	P3
Template (mmol)	Heptachlor (0.2)	Heptachlor (0.2)	Heptachlor (0.2)
Monomer (mmol)	MAA (1.0)	4-VP (1.0)	Styrene (1.0)
Cross-linker (mmol)	EDMA (5.0)	DVB (5.0)	DVB (5.0)

MAA - Methacrylic acid; EDMA - Ethylene glycol dimethacrylate

and heptachlor-co-Styrene-co-DVB (P3). Cross-linker and AIBN were then added subsequently, and the solution was ultrasonicated for 10 min to mix the contents thoroughly. The flask was purged repeatedly with pure nitrogen (purity is 99.99%) for 15 min to expel air and sealed immediately. The flask was heated for 24 h at 60°C to ensure complete cross-linking and polymerization. The resultant hard bulk polymers were ground mechanically and sieved to obtain particles of size less than 100 μm. The template was released by sonicating the ground polymer with methanol containing 10% acetic acid in a water bath sonicator till the extract phase showed nil template. The extracted heptachlor was detected by reverse phase HPLC. After complete extraction of template, polymers were washed with methanol to remove residual acetic acid. Finally, the MIPs were dried at 60°C for 24 hrs before rebinding experiments.

Polymer evaluation using binding experiments

The standard solutions of heptachlor were prepared in chloroform, and a calibration curve was obtained between the area and concentration of heptachlor using reverse phase HPLC. Twenty mg of the imprinted polymer was weighed in tubes, and 5 ml solution of heptachlor of known concentration was added to the tubes. The mixture was shaken for 24 hours on a horizontal shaker. Supernatant was filtered and injected to HPLC. The amount of free heptachlor was quantitiated using the calibration curve. Q_{max} was calculated using the following formula.

Q MIP (g) = μmol (heptachlor bound)

$$\frac{(C_i - C_f) V_s \times 1000}{M_{MIP}}$$

Where Q is binding capacity of MIPs (μmol/g), C_i the initial heptachlor concentration (μmol/ml), C_f the final heptachlor concentration (μmol/ml), Vs the volume of solution tested (ml), M_{MIP} the mass of dried polymer (mg).

Results and Discussion

Interaction between template and functional monomers

For the formation of a good imprinted polymer, it is important that there is adequate amount of interaction between the functional monomer and the template. The reaction mixture before the polymerization was investigated. The study was performed for seeing the interactions between template heptachlor and functional monomer MAA. We need to choose a solvent, which does not interfere with the hydrogen bonding between the template and the functional monomer. Further, the solvent should have different absorbance λ- max for the template and functional monomer under study. As the λ- max for heptachlor in chloroform is 245 nm and for MAA it is 265 nm, chloroform is a solvent of choice. The observation proposes

the formation of hydrogen bonding between the hydrogen of cyclopentadiene ring of heptachlor and the carboxyl of –COOH group of MAA. The proposed bond could be of C-O-H type, which is possible between the MAA and heptachlor molecule. Figure 2 shows that as the concentrations of MAA were increased in solution, the peak of heptachlor showed red shifts, and the corresponding maximum absorbance also decreased gradually. The red shift shown was not very peculiar for all the increasing concentrations, but the corresponding absorption decreased gradually. This effect is seen when hydrogen bonding effects π-π* absorption band of a molecule, whose chromophore acts as a proton donor.

A complex reaction of template (A) with functional monomer (B) can be described by the following reaction:

A + nB=C   (1)

For a MAA analytical concentration (b_0) greater than that of heptachlor (a_0), the complex concentration can be calculated according to:

$c = a_0 b_0^n k/(1 + b_0^n K)$ (2)

Where K refers to the association constant, n=1, 2, 3.....

The absorbance measured at a wavelength where b_0 does not absorb is:

$A = [(a_0 - c)\varepsilon_A + c\varepsilon_C] l$ (3)
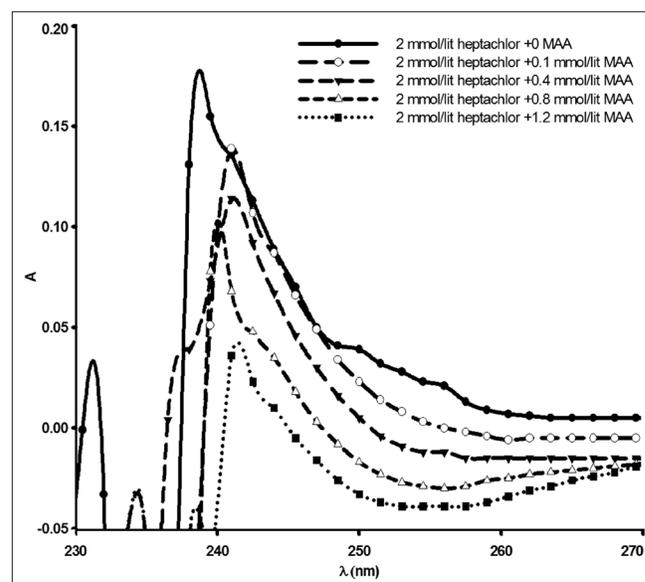
Figure 2: UV spectra of heptachlor in the presence of various concentration of MAA in dry chloroform. Concentration of heptachlor: 0.2 mmol l⁻¹; concentration of MAA for lines 1-5: 0, 0.1, 0.4, 0.8, 1.2 mmol l⁻¹; corresponding pure MAA solutions as blanks

Where ε_A and ε_C are the molar absorptivities of A and C, respectively. For $b_0=0$ the absorbance is:

$$A_0=a_0\varepsilon_A l \qquad (4)$$

The absorbance difference measured is:

$$\Delta A = A - A_0 = c\Delta\varepsilon l \qquad (5)$$

Where $\Delta\varepsilon = \varepsilon_C - \varepsilon_A$, substituting Eq. (5) into Eq. (2) yields:

$$\Delta A/b0^n = -K\Delta A + K\Delta\varepsilon a0l$$

K may be obtained by plotting $\Delta A/b0^n$ vs. ΔA.

When $\Delta A/b_0^n$ was plotted on X axis and ΔA on Y axis, the line obtained was linear at $n=1$ (which in the no. of interaction sites) as shown in Figure 3. K is calculated to be to be 0.551×10^3 M^{-1} from its slope. This shows that there is 1:1 complex formation between heptachlor and MAA. These pre-polymerization studies show that there in formation of a complex between MAA and heptachlor, and a good imprint can be formed after polymerization.

Binding capacity of the MIPs

Binding capacity was studied for all 3 polymers heptachlor-co-MAA-co-EDMA, heptachlor-co-4-VP-co-DVB, and heptachlor-co-styrene-co-DVB. The imprinting factors of the polymer for heptachlor in heptachlor-co-MAA-co-EDMA were found to be 1.9 followed by heptachlor-co-4-VP-co-DVB, which was 1.48. Heptachlor-co-styrene-co-DVB showed least imprinting effect that was 1.24 [Table 2]. It can be seen from the structure and interactions of the molecules that heptachlor can form H- bonding with MAA that leads to a good binding. However, in case of 4-VP and styrene, only other weak interactions like electrostatic, π-π and van der Waals interactions are possible between the monomer and the template and to some extent steric effects may contribute. The results are supported by the above experiment showing the interactions between heptachlor and MAA. The results were also supported with computational experiments done using the above 3 template monomer combinations, and the results were found in accordance with the experimental results.[35] The polymers were also successfully used for molecularly imprinted solid phase extraction of heptachlor from soil samples.

Effect of solvents

The binding of template to polymer depends to a great extent to the solvent used for the binding. Solvents create the atmosphere for the template binding. Generally, the solvent used as porogen is the best solvent for binding studies. Chloroform and methanol have been used in the present study. Binding of heptachlor in chloroform was 35.2 μmol g^{-1} of dry polymer and 29.08 μmol g^{-1} in methanol [Figure 4]. The non-specific binding to non-imprinted polymer (NIP) was also decreased. In chloroform, the amount of heptachlor
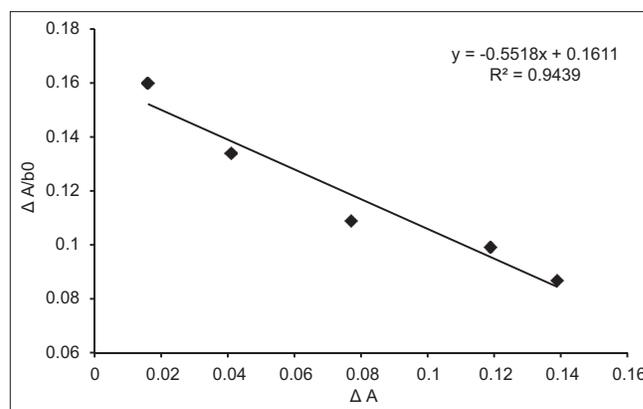
Figure 3: Plot of $\Delta A/b_0$ vs. ΔA for heptachlor interaction with MAA in dry chloroform
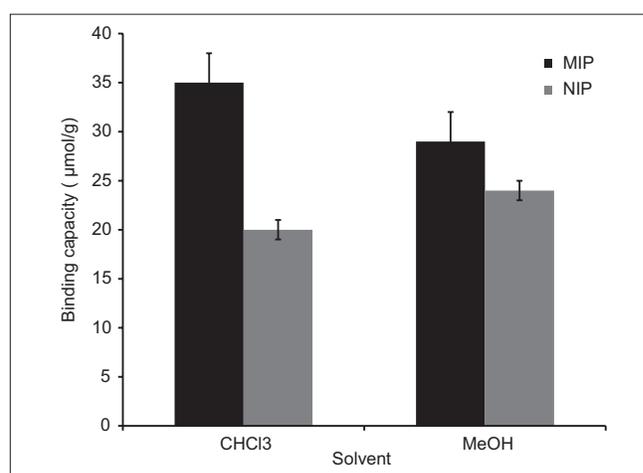
Figure 4: Binding of heptachlor to MAA-co-EDMA polymer in chloroform and methanol. Error bars represent±standard deviation on measurements made in triplicate

Table 2: Comparison of maximum rebinding amount for different molecular imprinted polymers and non-imprinted polymers

Code	Qmax±R.S.D (μg/g) ($n=3$)	Q_{MIPs}/Q_{NIPs}	Q_{MIPs}/Q_{NIPs}
P1	14.9±3.7	7.7±2.6	1.92
P2	15.5±3.4	10.5±0.68	1.48
P3	9.8±2.7	7.9±2.1	1.24

Polymers weight: 20 mg; solvent: dry chloroform; heptachlor concentration: 0.2 mmol/lit; volume of standard solution: 5 ml; incubation time: 24 h

bound to polymer was 20 μmol g^{-1}, which was less compared to 24 μmol g^{-1} of heptachlor bound to NIP in methanol. This could be due to the interference in H-bonding of heptachlor to polymer, which is reduced in the presence of chloroform. Chloroform being non-polar in nature does not interfere with the binding.

Selectivity for analogs

The polymer prepared using MAA as functional monomer

and EDMA as cross-linker was tested for the selectivity for analogous compounds endosulfan and heptachlor epoxide. The batch rebinding method was used for the study and was done same as done for heptachlor binding capacity. The imprinting factor for endosulfan was obtained to be 1.51±2.5 and for heptachlor epoxide it was 1.49±1.9. It shows that the polymer was more selective for heptachlor and can be used for selective extraction of the compound from complex environmental matrices.

Conclusions

Molecular imprinted polymers were successfully prepared for heptachlor using MAA and EDMA as functional and 2 cross-linking monomers. Hydrogen bonding between heptachlor and MAA was investigated with UV spectrophotometric studies. The selectivity for other 2 polymers with 4-VP (1.48) and styrene (1.24) were less compared to the one formed with MAA (1.92). The polymers have been applied for solid phase extraction, and satisfactory recoveries were obtained for the samples, suggesting that these MIPs can be applied to the sample preparation method.

References

1. Mosbach K, Haupt K, Liu XC, Cormack Peter AG. Ramstrom O. Molecular Imprinting: *Status Artis et Quo Vadere?* in Molecular and Ionic Recognition with Imprinted Polymers. Am Chem Soc 1998;703:29-48.

2. Arnold BR, Euler AC, Jenkins AL, Uy OM, Murray GM. Progress in the Development of Molecularly Imprinted Polymer Sensors. Johns Hopkins APL Tech Digest 1999;20:190-8.

3. Haupt K. Molecularly imprinted sorbent assays and the use of non-related probes. Reactive and functional polymers 1999;41:125-31.

4. Haupt K, Mosbach K. Plastic antibodies: Developments and applications. Trends Biotechnol 1998;16:468-75.

5. Jenkins AL, Bae SY. Molecularly imprinted polymers for chemical agent detection in multiple water matrices. Analytica Chimica Acta 2005;542:32-7.

6. Jenkins AL, Yin R, Jensen JL. Molecularly imprinted polymer sensors for pesticide and insecticide detection in water. Analyst 2001;126:798-802.

7. Kriz D, Ramström O, Mosbach K. Molecular Imprinting: New Possibilities for Sensor Technology. Anal Chem 1997;69:345A- 9A.

8. Lübke M, Whitcombe MJ, Vulfson EN. A Novel Approach to the Molecular Imprinting of Polychlorinated Aromatic Compounds. J Am Chem Soc 1998;120:13342-8.

9. Müller R, Andersson LI, Mosbach K. Molecularly imprinted polymers facilitating a β-elimination reaction. Die Makromolekulare Chemie Rapid Commun 1993;14:637-41.

10. Hennion MC, Pichon V. Immuno-based sample preparation for trace analysis. J Chromatogr A 2003;1000:29-52.

11. Pichon V, Delaunary-Bertoncini N, Hennion MC. In Sampling and Sample Preparation for Field and Laboratory (Comprehensive Analytical Chemistry) Vol. 37. In: Pawliszyn J, editor. Amsterdam: Elsevier Science; 2002. p. 1081-100.

12. Caro E, Marcé RM, Borrull F, Cormack Peter AG, Sherrington DC. Application of molecularly imprinted polymers to solid-phase extraction of compounds from environmental and biological samples. TrAC Trends Anal Chem 2006;25:143-54.

13. Lanza F, Sellergren B. Molecularly imprinted extraction materials for highly selective sample cleanup and analyte enrichment. Adv Chromatogr 2001;41:137-73.

14. Siemann M, Andersson LI, Mosbach K. Selective recognition of the herbicide atrazine by noncovalent molecularly imprinted polymers. J Agric Food Chem 1996;44:141-5.

15. Baggiani C, Anfossi L, Giovannoli C, Tozzi C. Binding properties of 2,4,5-trichlorophenoxyacetic acid-imprinted polymers prepared with different molar ratios between template and functional monomer. Talanta 2004;62:1029-34.

16. Han DM, Fang GZ, Yan XP. Preparation and evaluation of a molecularly imprinted sol-gel material for on-line solid-phase extraction coupled with high performance liquid chromatography for the determination of trace pentachlorophenol in water samples. J Chromatogr A 2005;1100:131-6.

17. Meng Z, Yamazaki T, Sode K. Enhancement of the catalytic activity of an artificial phosphotriesterase using a molecular imprinting technique. Biotechnol Lett 2003;25:1075-80.

18. Zhu X, Yang J, Su Q, Cai J, Gao Y. Selective solid-phase extraction using molecularly imprinted polymer for the analysis of polar organophosphorus pesticides in water and soil samples. J Chromatogr A 2005;1092:161-9.

19. Graham AL, Carlson CA, Edmiston PL. Development and Characterization of Molecularly Imprinted Sol-Gel Materials for the Selective Detection of DDT. Anal Chem 2001;74:458-67.

20. Dickert FL, Achatz P, Halikias K. Double molecular imprinting--a new sensor concept for improving selectivity in the detection of polycyclic aromatic hydrocarbons (PAHs) in water. Fresenius J Anal Chem 2001;371:11-5.

21. Dickert FL, Tortschanoff M, Bulst WE, Fischerauer G. Molecularly Imprinted Sensor Layers for the Detection of Polycyclic Aromatic Hydrocarbons in Water. Anal Chem 1999;71:4559-63.

22. Lai JP, Niessner R, Knopp D. Anal Chim Acta 2004;522:137-44.

23. Davidow B, Radomski JL. Isolation of an epoxide metabolite from fat tissues of dogs fed heptachlor. J Pharmacol Exp Ther 1953;107:359-65.

24. Ahmad R, Salem NM, Estaitieh H. Occurrence of organochlorine pesticide residues in eggs, chicken and meat in Jordan. Chemosphere 2010;78:667-71.

25. Bhuiyan NI, Bhuiyan HR, Nath KK. Incidence of organochlorine insecticides (DDT and heptachlor) in Bangladeshi dry fish: Seasonal trends and species variability. Afr J Environ Sci Technol 2009;3:405-11.

26. Bulut S, Erdogmus SF, Konuk M, Cemek M. The Organochlorine Pesticide Residues in the Drinking Waters of Afyonkarahisar, Turkey. Ekoloji 2010;19:24-31.

27. Gunderson EL. Dietary intakes of pesticides, selected elements, and other chemicals: FDA Total Diet Study, June 1984-April 1986. J AOAC Int 1995;78:910-21.

28. Shegunova P, Klanova J, Holoubek I. Residues of organochlorinated pesticides in soils from the Czech Republic. Environ Pollut 2007;146:257-61.

29. Tanabe S, Kannan K, Tabucanon MS, Siriwong C, Ambe Y, Tatsukawa R. Organochlorine Pesticide and Polychlorinated Biphenyl Residues in Foodstuffs from Bangkok. Thailand. Environ Pollut 1991;72:191-203.

30. Hrdina PD, Peters DA, Singhal RL. Effects of chronic exposure to cadmium, lead and mercury of brain biogenic amines in the rat. Res Commun Chem Pathol Pharmacol 1976;15:483-93.

31. Krampl V. Relationship between serum enzymes and histological changes in liver after administration of heptachlor in the rat. Bull Environ Contam Toxicol 1971;5:529-36.

32. Reuber MD. Histopathology of carcinomas of the liver in mice ingesting heptachlor or heptachlor epoxide. Exp Cell Biol 1977;45:147-57.

33. Yamaguchi I, Matsumura F, Kadous AA. Pestic Biochem Physiol 1979;11:285-93.

34. Williams GM, Numoto S. Promotion of mouse liver neoplasms by the organochlorine pesticides chlordane and heptachlor in comparison to dichlorodiphenyltrichloroethane. Carcinogenesis 1984;5:1689-96.

35. Singh K, Balasubramanian S. Amitha Rani BE. Computational and Experimental Studies of Molecularly Imprinted Polymers for Organochlorine Pesticides Heptachlor and DDT. Curr Anal Chem 2012;8:562-68.