

# BIODEGRADATION OF XENOBIOTIC COMPOUNDS

## Abstract

Xenobiotic compounds are chemical compounds that are produced by man and by the environment also but in very low concentrations. If these compounds are getting accumulated in the environment, then they will reach toxic concentrations and gives harmful effectson our environment. We need to reduceits direct or indirect use in our day-to-day life. We should also think about its degradation so that it won't get accumulated in our environment. For doing this we should first know about it, its chemical nature, its degradability according to its chemical composition, its biodegradation by different microorganisms and the metabolic pathways within the microorganism, and the metabolic complications which arise because of the complexity of the compounds.All the above information will help us to boost the existing metabolic pathways of microorganisms and also to genetically modify the metabolic pathways to keep our environment somewhat clean and healthy. here we are going to discuss all the related information like-what is the basic meaning of xenobiotic compounds, their effect on metabolic pathways of a microorganism according to its chemical composition like the presence of halogens, cyclic groups, branched-chain, etc., hazards from xenobiotic compounds like toxicity, carcinogenicity, etc. After knowing its chemical composition and hazardous effect we will discuss the bacteria which are involved in the biodegradation of these compounds and the general features of its biodegradation, then we will discuss the co-metabolism and gratuitous metabolism of these compounds by microorganisms this information on co-metabolism will help us to boost the metabolic pathways involved, then we will discuss little the origin and capacity to degrade xenobiotic compounds because of mutation and plasmid transfer among the bacteria. After gathering all of this

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information we will discuss the practical approaches to degrade these xenobiotic compounds like using mixed culture etc.

**Keywords:** Xenobiotic compounds, complexity, biodegrading pathways, metabolism, co-metabolism

## I. INTRODUCTION

What are xenobiotic compounds?

Xenobiotic compounds are man-made chemicals and are also produced by environment, but the amount produced by the environment is very low as compare to ours.[6] Xenobiotics have been defined as chemicals to which an organism is exposed that are extrinsic to its normal metabolism. Without metabolism, many xenobiotics would reach toxic concentrations which are harmful to the environment. Most metabolic activity inside the cell requires energy, cofactors, and enzymes to degrade these compounds.[2] A xenobiotic is a chemical that is not used by the reference organism as a nutrient chemical, is not essential to the reference organism for the maintenance of normal physiologic or biochemical function and homeostasis, and does not constitute a part of the conventional process of chemicals synthesized from nutrient chemicals by that organism in normal intermediary metabolism.[3] Here we will come to see how these xenobiotic compounds are degraded in the environment, their effect on the environment, how important the microorganisms are in the degradation of these compounds, and how the compound's complexity affects their metabolism inside bacterial cells[7]

## II. METABOLIC EFFECTS OF XENOBIOTICS ON MICROORGANISMS

In 1965 Alexander has given a principle called microbial infallibility which says that microorganism can degrade all compounds occurring naturally.[1]

Many of the xenobiotic compounds are degraded by microorganism, but are not capable of degrading all of them. The compounds that resist biodegradation by these microorganisms and therefore persist in the environment for a long time are called recalcitrant.[31]

### 1. The xenobiotic compounds may persist for a long time because of the following reasons:

- They are not recognized as a substrate by the degradative enzymes which are already present in the environment,
- Substitution groups such as halogens, amino-, sulphonate, nitro-, methoxy- and carbamoyl groups, or (He, Ne, AR, Kr, Xe, Rn) make the compound hard to degrade that is biologically and chemically inert.
- They may be insoluble in water naturally in the environment, or are adsorbed to external matrices like soil,
- They are highly toxic or may give rise to toxic products because of microbial activity present naturally in the environment
- They may have a large size that hinders their entry inside the microorganism cell,
- Xenobiotic compounds may be unable to induce the synthesis of enzymes involved in degrading, and
- Lack of the permease enzyme used for compounds to be transported into the microbial cells.
- Some oil mixture and Some others.[8]

**2. There are some structural complications also that make these compounds hard to be degraded by the microorganisms, these are as follows:**

- (Hydrogen of the molecule may be replaced by halogens; or the carbon-halogen(Cl,F,etc.) bond which may be hard to be degraded and if cleaved then it uses a high amount of energy[32],
- Presence of other groups like nitro-, sulphonate, amino-, methoxy-, and carboxyl groups in place of H, change the compound in such a way which are not degraded easily,
- Linear chain or aliphatic compounds are easy to degrade while presence of aromatic and cyclic compound is more arrogant to degrade and not just these but presence of cycloalkanes and heterocyclic compounds are also hard to degrade.
- Branched linear chains are also not easily degraded etc..In the environment, the complexity of the xenobiotic compound is directly linked with its complex digestion. The large molecular size, water insolubility make them resistant to biodegradation so many of them remains undigested in environment, as discussed earlier[9].
- Here also hydrogen atom gets replaced with various atoms like Cl, Br, F, I, etc. (Halogens). These are used in our day-to-day life as solvents as chloroform, as propellants in spray cans of cosmetics, paints,etc., in condenser units of cooling systems (CCl<sub>3</sub>F, CCl<sub>2</sub>F<sub>2</sub>, CClF<sub>3</sub>, CF<sub>4</sub>), and as insecticides used to kill insects (DDT, BHC, lindane, etc.) and herbicides to kill herbs in farms (dilation, 2, 4-D, 2, 4, 5-T, etc.)[10].

The chloroforms and freons are C<sub>1</sub>-C<sub>2</sub>haloalkanes which can escape into atmosphere as they are volatile and after mixing into atmosphere they lead to increased UV radiation as they disrupt the O<sub>3</sub> i.e. protective ozone covering of Earth. They also get reach to water bodies as we use pesticides for our crops from where they get mixed into it;and this leads to bio-magnification which also affects the bird egg.[11]

- **Polychlorinated Biphenyls (PCBs):** These compounds have two covalently linked benzene rings which have halogens substitution for Hydrogen. PCBs are mainly used as plasticizers, insulator coolants in transformers, and as heat exchange fluids,these are both biologically and chemically inert to various degrees, this inertness and arrogant nature is directly linked with the number of chlorine atoms present in the molecule[12]. The halogenation and cyclic structures (PCBs) of the above two groups make them more persistent [5].
- **Man-made Polymers:** These types of compounds such as plastics are mainly used as wrapping materials, in making garments, etc. The large molecular size and water insolubility make them to persist in nature and because of this they are not easily permitted in microbial cells. [33]
- **Alkylbenzyl Sulfonates:** These alkylbenzyl sulfonates are surface-active detergents superior to the soap that we use. These compounds affect the degradation in two ways -1) If sulphonate ( $\text{—SO}_3^-$ ) group is present even at single place then this type of compound hinders the biological degradation, 2) At other end non-polar alkyl end becomes recalcitrant if, the degree of branching become more. To overcome this,

nowadays alkylbenzyl sulphonates which have unbranched alkyl ends are used; these are easily biodegradable and are by doing  $\beta$ -oxidation starting from alkyl ends of the compounds.[5]

- **Mixture of different oils:** Oil is produced naturally, it has many components and is biodegradable, but since it has different components, these components get degraded at different rates. Biodegradation can degrade small amount of oil waste as nature can tolerate a limited amount of any such substances so, the occurrences of large number of spills create pollution problem. Oil is not soluble in water and are even toxic so it won't be degraded if present in higher amounts[13].
- **Other Xenobiotic Compounds:** Several pesticides are based on aliphatic, cyclic ring structures containing a substitution of nitro-, sulphonate, methoxy-, amino- and carboxyl groups; in addition, they also contain halogens and as written earlier these substitutions make them recalcitrant for nature and arrogant to be degraded by microorganisms. [5]

### III. HAZARDS FROM XENOBIOTIC COMPOUNDS

#### 1. The xenobiotics have several hazards to us and to different organisms also these are explained below.

- **Hazards:** In many xenobiotics H is replaced by halogen and aromatic hydrocarbons groups which are toxic to environment. At low concentrations they may cause various skin problems and reduce reproductive potential at higher concentrations they may cause more complexity[14].
- **Carcinogenicity:** Certain halogenated hydrocarbons are carcinogenic that is they can cause cancer
- Many xenobiotics are not degraded and remains in the environment for a long time so they increase in concentration with time[34].
- Xenobiotics like DDT and PCBs are not degraded easily; because of this arrogant nature, they show bioaccumulation or bio-magnification (often by a factor of 10<sup>4</sup> – 10<sup>6</sup>).[15].

#### 2. Bio-magnification occurs mainly because of the following two reasons:

- These arrogant compounds are continuously taken up from the environment and since they are not degradable, they accumulate in body, for example, accumulation of DDT by plankton from the water bodies, and from here they enter in the food chain[16].
- After entering in food chain, they are passed from one organism to another, e.g., plankton → small fish → large fish → sea-eagles; and this concentration increases as we step further in the food chain as it gets accumulated in every organism[17].
- In the case of sea eagles 10<sup>5</sup>- a fold increase of DDT occurs as because of which sea eagles laid fragile eggs. DDT and PCBs have been found in human tissues in high but sub-lethal concentrations in those countries where these chemicals are used, although humans were often not in direct contact with these chemicals, they come in indirect contact through the food chain[18].

- (They are produced and used in large quantities for our doings which favors their accumulation in nature.[1])

#### IV. BACTERIA INVOLVED IN THE DEGRADATION OF XENOBIOTIC COMPOUNDS

Microorganisms generally apply two modes of action for the degradation of this xenobiotic compounds –

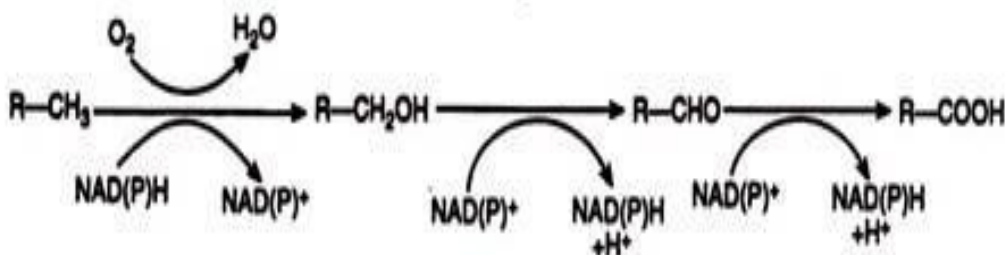
- Aerobic biodegradation;
  - Anaerobic biodegradation.
1. Examples of aerobic degradative bacteria of xenobiotics are as follows- *Pseudomonas*, *Gardenia*, *Bacillus*, *Moraxella*, *Micrococcus*, *Escherichia*, *Sphingobium*, *Pandoraea*, *Rhodococcus*, and anaerobic xenobioticsdegradative bacteria are *Pelatomaculum*, *Desulphovibrio*, *Methanospirillum*, *Methanosaeta* *Desulfotomaculum*, *Syntrophobacter*, *Syntrophus*.
  2. Anaerobic habitats, like sludgedigesters, groundwater, sediments, water-laden soils, gastrointestinalcontents, feedlot wastes and landfill sites (Williams, 1977) and somexenobiotic compounds (e.g., tetrachloroethylene, polychlorinatedbiphenyls (PCBs), and nitro-substituted aromatics) can be effectivelytransformed or mineralized by anaerobic bacteria.[19].

#### V. COMMON FEATURES OF XENOBIOTICS BIODEGRADATION

Since xenobiotics are made up of a large number of compounds, so the large number of metabolic pathways are involved in their degradation inside microbial cell[31].

##### 1. Alkanes and aromatic hydrocarbons aregenerally degradedthroughthe following:

- To make the compound more reactive hydroxyl group is first introduced by oxygenase,
- This hydroxyl group is then oxidized to a carboxyl group,
- In the case of the cyclic compound the ring structure is opened up first,
- (iv)While  $\beta$ -oxidation is used to degrade the linear molecule this yields acetyl CoA this is then metabolized in the usual manner, here we have shown the example of n-alkane: (Fig 1).[1].

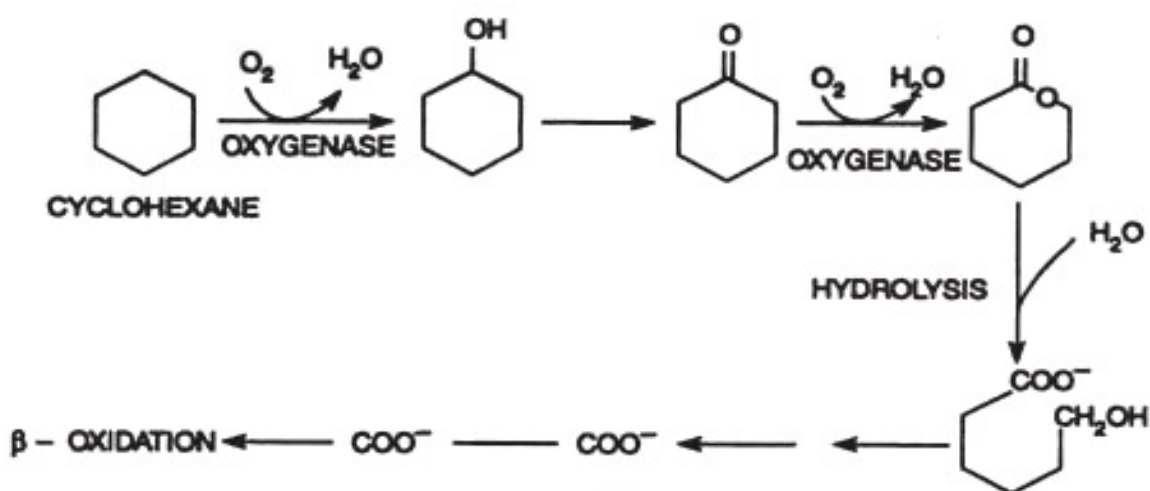


**Figure 1:** Degradation of alkanes and aromatic hydrocarbons.[1].

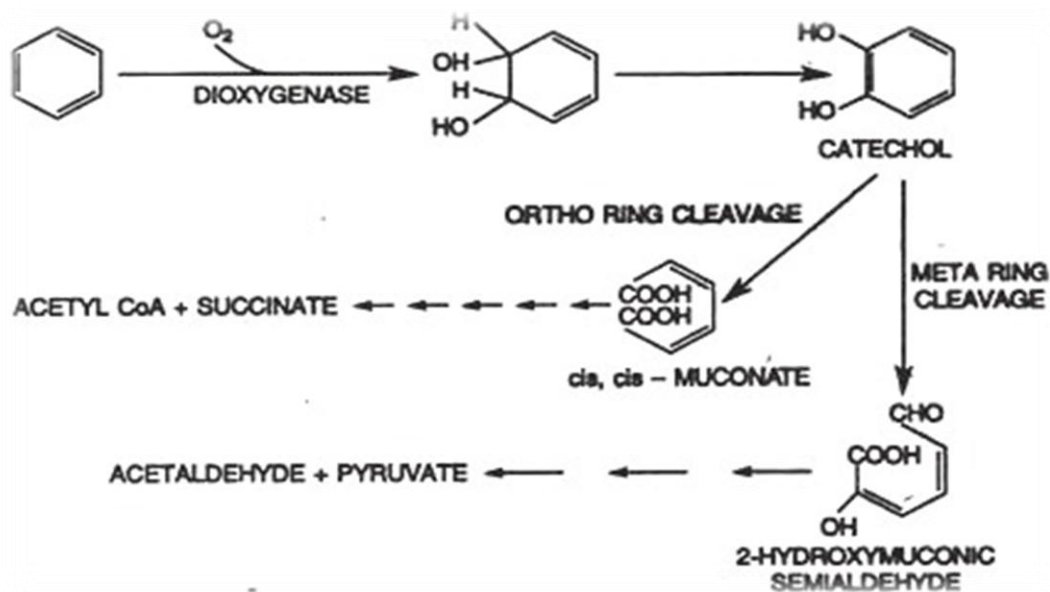
**2. An alicyclic hydrocarbon, is also degraded similarly for example; cyclohexane, is oxidized as shown here:**

- Firstly, an —OH group is added by oxygenase in the ring, to make it more reactive,
- Then an ester in the form of a lactone is formed by the oxygenase,
- This is then hydrolyzed to open the ring structure which yields a linear molecule, which can easily be degraded. (Fig.2)

Mono-oxygenases are involved in both of these oxidations which add oxygen to a single position in the molecule, while, oxidation of the benzene ring may involve a di-oxygenase which adds oxygen at two positions in the molecule in a single step. (Fig.3) [1].



**Figure 2:** Oxidation of cyclohexane. Two different monooxygenases are involved.



**Figure 3:** Oxidation of aromatic hydrocarbons initiated by the action of a dioxygenase.

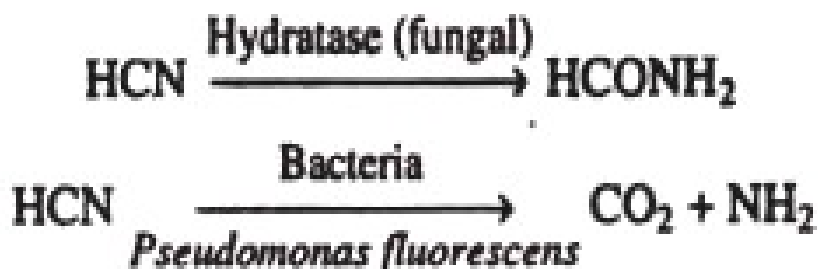
Mono- oxygenase and di-oxygenase are of different types: many of them react better with alkanes having short chains, while others react on alkanes having cyclic groups attached with it. Each enzyme oxidizes a limited range of compounds and these enzymes are not very specific in nature. Thus, a wide variety of microorganisms degrade Xenobiotic compounds, small range of compounds are degraded by each of them [20].

Xenobiotics oxidation employs cytochrome P450 or rubredoxin. In addition, in initial reaction the halogen or some other substituted groups are removed or even changed or modified discussed or sometimes it is done after few steps [1].

## VI. HYDROCARBON DEGRADATION

1. **Halomethanes:** The enzyme methane mono-oxygenase transfers Halomethanes into methanol which uses them as substrate; this enzyme occurs in several methylotrophs. Oxidative dichlorination of methanol from halomethanes occurs by glutathione-dependent hydrolase; this is an anaerobic reaction in nature. Oxidation of Methanol to CO<sub>2</sub> + H<sub>2</sub>O is done by formic acid and formaldehyde. [21]
2. **Cyanide:** HCN is harmful for environment but some microorganisms are able to degrade cyanide but in smaller amount. Cyanides, such as HCN and CH<sub>3</sub>CN, can get mixed with air. Therefore, we should minimize its use. [35]

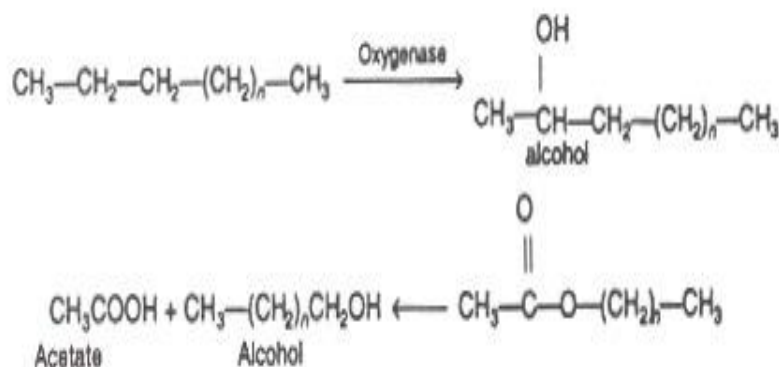
The degradation of cyanides is shown here:



- **Hydrocarbons which are aliphatic in nature:** Saturated or unsaturated aliphatic hydrocarbon, n-Alkanes of 10-24 carbons are most easily degraded by microorganisms. Similarly, like aliphatic hydrocarbon, saturated aliphatic is degraded more easily than hydrocarbon having double or triple bond while decreased biodegradation is observed in case of branched chains. Oxygenase's n-alkane biodegradation of n-alkanes gives carboxylic acid which is then degraded by β-oxidation. [22]

Methane group involve oxidation at one end of the n-alkane molecule, or at a β-methylene group (Fig. 4) the oxidation may occur. Dicarboxylic acid is produced when both terminal methyl groups are oxidized, the biodegradation of branched-chain n-alkanes may take place by the above reaction in case of many biodegrading cells [1].





**Figure 4:** Oxidation of b-methylene group of an n-alkane.

### 3. Metabolism of Xenobiotic Compounds by Microorganisms and co-metabolism involved:

- Some xenobiotic compounds, are degraded by microbes, but are not used as sources of carbon and energy by these microorganisms some of these compounds are halogenated[23].
- The presence of other compounds, like enzymes involved, reducing equivalent, energy etc. decides the biodegradation of these types of compounds. Presence of co-metabolite and metabolic pathway decides the degradation of these types of xenobiotic compounds.[24].
- Degradation of some xenobiotic compounds are done by existing metabolic pathway and by gratuitous metabolism in which reducing power and main source of energy is xenobiotic compounds; called gratuitous metabolism[30].
- In such a metabolism, necessary enzymes are not needed as co-metabolite and are induced by another compounds present.
- There is high extent of similarity between the natural substrate needed for enzyme needed in their degradation and xenobiotics degraded by co-metabolism[38].
- Generally, the complete degradation of xenobiotic compound is not done by the degrading cells but the product which is produced by this primary degradation is may be used as substrate by many bacteria or may be less polluting for the environment. [4].

## VII. THE ORIGIN OF CAPACITY TO DEGRADE XENOBIOTICS

Because of continued exposure of microorganisms to xenobiotic compounds cells may evolve new metabolic pathway which degrade the xenobiotic compound completely[25].

### 1. These capabilities may arise due to:

- Genetic mutation.
- Transfer or exchange of plasmid carrying such degrading protein genes.[36].  
Manmade Approaches to degrade xenobiotic compounds
- Concentration of xenobiotic compounds plays an important role in its degradation either it is low or high (toohigh concentration may be toxic and not degraded by

microorganisms), it also depends upon pH of the medium, its temperature, other nutrition and water availability.

- Use of xenobiotic compounds should be in very less amount so that it won't reach toxic concentration[29].
- In a sludge treatment tank, a continuous supply of such compounds should be done so that selective maintenance of such microbes capable of degrading such compounds should occur[26].
- Plus, the interfering organic compounds should not be present in the environment, as they can be degraded easily and also hinder the degradation of the main xenobiotic compound[28].

## **2. Manmade or practical approaches to increase the rate of xenobiotic degradation are as follows:**

- Supply of good amount of nutrients or co-metabolites should be done,
- Such toxic compounds should be maintained at low levels, and
- Provision of microbial population or inoculum [4].

## **3. Mixed Populations should be used:**

- The reason why we should use microbial mixed population for the degradation is as follows:
- Two different microbes can complement each other and degrade a xenobiotic wholly while, if they are present in a single, they may not be able to do this [37]. In such a case, the product of degradation by one microorganism may serve as the substrate for the other.
- Other microorganism may use the product made by first microorganism as their growth factor/nutrient mixed with it. This can be seen in *Nocardia* sp. which degrades cyclohexane but is unable to produce biotin. A *Pseudomonas* sp. strain produces biotin but cannot degrade cyclohexane these both are mixed to get their proper use [27].
- Plasmid transfer may occur fast in co-culture into a faster-growing species which therefore creates a new colony and thus biodegrade more and more xenobiotic compounds. [4].

## **VIII. CONCLUSION**

From the above discussion, we conclude that metabolism and co metabolism has an important effect on the biodegradation of xenobiotic compounds.

So, microorganisms can be used in waste treatment, biodegradation, pollution control, biomass energy production, etc.

The important aspect is to identify one by one the key metabolic reactions and the stable end product so that we come to know the necessary information to understand the processes, tendencies, and role of microorganisms in altering the character of the important group of environmental pollutants like Xenobiotics. If we come to know the end products and metabolic pathways used by microorganisms then it will become easy to use genetic strategy to modify the microorganism involved in degradation. Many different factors also affect the

biodegrading strategy of microorganisms, these are as follows-Soil moisture, Organic and inorganic chemicals (also including chemicals produced during metabolism, Soil organic matter, Types of vegetation, and microorganism's growth strategy. So, biodegradation done by microorganisms is mainly-A primary degradation, in which the characteristic property of the Xenobiotic compound gets disappeared; and is converted into an environmentally acceptable substance. Biodegradation is the minimum alteration of the parent compound that is only necessary to remove properties occurs; and in ultimate biodegradation, the complete conversion of the parent compound to the inorganic end-products which are associated with the microorganism's normal metabolic processes occurs. There are many direct and indirect sources of xenobiotic compounds -Direct Sources-Plastic, paints, phenolic compounds, petroleum products, dyes, and pigment Indirect sources- are pharmacy products like drugs, fertilizer, etc., So we should reduce the use of these products and should replace these products with some healthy substitute.

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