Report Summary:

The National Institute of Health (NIH shift) is a chemical shift of substituents in chemical reactions named after the founders who first observed and reported this 1, 2 rearrangement. This chemical shift of substituents usually involves aromatic compounds but has also been observed in nonaromatic hydroxylation reactions. The substituents that undergo this shift are hydrogen, deuterium, halogens, acyl, aryl and alkyl groups. It is an important aspect that is a requirement in the hydroxylation of aromatic compounds by monooxygenase enzymes. In the hydroxylation of aromatic compounds the substituents undergo a 1, 2 migration reaction also known as the NIH shift, where the transfer of an oxygen atom in the monooxygenase enzyme catalyzed reaction is electrophilic, and the substituents that participate in this mechanism activate the aromatic ring toward an electrophilic attack. Deactivating groups consist of electron withdrawing groups that activate the aromatic ring towards an electrophilic attack. These groups are classified as weak, moderate, and strong deactivating groups that attach to a ring and allows for the removal of electron density from the aromatic ring. The weak deactivating groups direct the electrophiles to attack the benzene molecule at the ortho and para positions on the ring. On the other hand, strongly and moderately deactivating groups direct attacks to the meta position of the aromatic ring. Dr. Kumar has shown an acyl migration in an intramolecular NIH shift study at Governors State University. In this literature search I have examine the NIH shift in different aspects in order to build on and give insight into Dr. Kumar’s study on the subject and its mechanism in different hydroxylation reactions. In this report the NIH shift and its involvement in hydroxylation reactions will be studied and reported in terms of there involvement in different reactions. I began my literature
search on the years following Dr. Kumar’s research on the subject. The substituents are radiolabel for tracking the site and retention of deuterium in hydroxylation reactions. Hydroxylation is a chemical process that introduces a hydroxyl group into an organic compound. This is the first step in the oxidative degradation of organic compounds in air and important in detoxification since hydroxylation converts lipophilic compounds into hydrophilic products that are more readily excreted, and some drugs are activated or deactivated by hydroxylation. The hydroxylases are any of a group of enzymes that catalyze the formation of a hydroxyl group on a substrate by incorporation of one atom or two atoms of oxygen from dioxygen. They all are homotetrameric, contain a mononuclear iron and utilize dioxygen and tetrahydrobiopterin as substrates in these reactions.

In the hydroxylation reaction above a hydroxylase adds a hydroxyl group to the ring, and the labeled site shifts one position around the aromatic ring relative to the stationary methyl group which is the 1, 2 migration known as the NIH shift.

Monooxygenase are oxidoreductase enzymes that incorporate one hydroxyl group into substrates in metabolic pathways, they also participate in the catalysis of electron transfer reactions. In this reaction, two atoms of oxygen are reduced to one hydroxyl molecule.
and one water molecule by concurrent oxidation. The electrophilic aromatic substitution mechanism is also studied in terms of the NIH shift in these articles because this shift is often indicative of their existence. Electrophilic aromatic substitution involves the substitution of an electrophile with a hydrogen atom on the aromatic ring. Certain groups can be directly introduced onto the ring in the presence of a catalyst the alkyl/acyl, halogens, nitro & sulfonic acid groups. When the transition states for hydroxylation mechanisms prove to be highly electron deficient, this is consistent with production of a cationic intermediate, proving electrophilic aromatic substitution. The size and regioselectivity of the substituents often affects hydroxylation and the NIH shift mechanism is used to identify these entities. There are three pterin dependent hydroxylases that share a common function; during hydroxylation of an amino acid an NIH shift occurs prior to oxygen addition to the ring. Tyrosine hydroxylase catalyzes the conversion of tyrosine to dihydroxyphenylalanine using molecular oxygen and tetrahydrobiopterin in scheme 1 below. This is the rate limiting step in the biosynthesis of the catecholamine neurotransmitters dopamine, epinephrine and norepinephrine.

![Scheme 1](image)

Each of these enzymes catalyze the hydroxylation of a specific aromatic amino acid, requires ferrous iron for activity, and has tetrahydrobiopterin and molecular oxygen as the other substrates. The function of tryptophan hydroxylase is to hydroxylate tryptophan
to form 5-hydroxytryptophan (Scheme 1). In mammalian metabolism this is the rate-limiting process in the production of the neurotransmitter serotonin.

Scheme 1

More studies have been done on phenylalanine and tyrosine oppose to tryptophan due to its lack of resources until recently, the advent of a stable truncated rabbit form of tryptophan hydroxylase with only the amino acids of the catalytic domain, has afforded the opportunity to investigate this enzyme further. The researchers in the articles attempt to answer some questions regarding this enzymes mechanism by identifying the hydroxylating intermediate, the rate limiting step in catalysis, and the addition of oxygen to the ring of the amino acids with the NIH shift in the catalytic mechanism of tryptophan hydroxylase.

Halogens are weakly deactivating ortho, para directing substituents with addition of a halogen to the ring (chlorination/bromination) requiring an acid catalyst such as ferric chloride/ aluminum chloride to form a strong electrophile (e.g., chloronium). When the strong electrophile attacks the ring a cationic intermediate forms with the positive charge on the ortho and para positions. The loss of a proton from the cation intermediate regenerates the acid catalyst and the resultant products. There were several articles involving the halogen substituents, including the fluorine and iodide substituents. In article #1 when tyrosine hydroxylase is used the fluorine substituent has a zero NIH shift value and thus only tyrosine is the resultant product. The
chlorine substrate has the next highest NIH shift value (0.22) and three products were formed with hydroxylation on either the 3\textsuperscript{rd} or 4\textsuperscript{th} carbon on the ring. The highest NIH value is found when bromine is the substrate (0.61), there are three products formed with hydroxylation occurring on either the 3\textsuperscript{rd} or the 4\textsuperscript{th} position on the ring. Tyrosine formed as a product for all three halogen substrates. The results suggest that an NIH shift occurs with the bromine and chlorine ion that’s consistent with a cationic intermediate. Article #11 provides evidence that the product of the monohalogenated benzenes except for fluorobenzene results in para-phenols and the only ortho product observed was from the fluorine substrate. There were some meta phenols formed from the halogens but the para phenols values were higher and thus makes them the primary products. The NIH shift value for all of the monohalogenated benzene substrates are very similar indicating a common intermediate when the deuterium is at the site of hydroxylation or an adjacent site. Iodobenzene has values less than (0.1) umols at both para and meta positions indicating no product formation for this halogen. The methoxy and alcohol substrates are ortho, para directing strongly activating substituents that delocalize the positive charge of the cationic intermediate while lowering its activation of energy for its formation, with an activating effect towards further substitution. There was a zero NIH shift for the methoxy substrate and only one product formed with hydroxylation occurring at the 3\textsuperscript{rd} carbon due to its larger size, hydroxylation favors the 3\textsuperscript{rd} carbon when the size of the substituent is large. When 4 X substituted phenylalalanines were used as substrates for tyrosine hydroxylase, several hydroxylated products formed and oxygen addition to the ring results in elimination of the X-substituent from the 4\textsuperscript{th} carbon or an NIH shift to form the products tyrosine and 3-X-tyrosine, respectively. The size of the X substituent on the 4\textsuperscript{th}
position of the ring determines the site of hydroxylation, with hydroxylation occurring on the adjacent 3\textsuperscript{rd} carbon when the substituents size is large, suggesting sterics is a primary determinant of the initial site of attack. The products that arise from a NIH shift decreases in the order: bromine with the highest shift, methyl and chlorine the next highest values, and methoxy and fluorine with zero NIH shift values.

The nonaromatic NIH shift in article #1 is consistent with the P450-monoxygenase mechanism supporting the iron oxygen theory. The relatively high negative roe values are due to an electron deficient transition state showing that attack of the hydroxylating intermediate forms a cationic intermediate. An excess of hydroxypterin over the hydroxylated amino acid (dihydroxyphenylalanine) when tyrosine is the substrate for tryptophan hydroxylase provides evidence of a hydroxylating intermediate that contains only one oxygen atom in article #4. There is an inverse isotope effect and an electron deficient transition state that provides proof of the presence of a cationic intermediate in all articles for the tyrosine and tryptophan hydroxylases. Article #1 showed an increase in the Km values due to the increase in the size of para substituents, proving a restricted active site in the area of this substituent when tyrosine hydroxylase is used. The hydroxylating species discriminates between carbons 4 & 6 in response to substrate atomic charge and the hydroxylation is less efficient at carbons 4 & 5 of the indole ring.

Nitration is an electrophilic aromatic substitution that involves the generation of the nitronium ion from the reaction of sulfuric and nitric acids. The nitronium ion attacks the benzene ring giving a cationic intermediate. The proton transfer from the resultant cationic intermediate to water or the sulfuric ion regenerates the ring and gives the product nitrobenzene. Sulfonation is similar to nitration utilizing the sulfuric acid that
contains sulfur trioxide instead of nitric acid. The nitro compound is a strongly deactivating meta directing substituent, while the methylsufonyl and carbon nitrate substituents are moderately deactivating meta directing substituents that decrease electron density on the ring and deactivates the ring to further substitution.

Article #11 examines the catalysis of ammonia monooxygenase enzyme and the migration of deuterium and hydrogen in aromatic hydroxylations that are specifically deuterated monosubstituted benzenes. There are low deuterium retention values in the oxidation of nitrobenzene to meta-phenols when the site for hydroxylation is deuterated, rather than when the retention is at other positions. A direct loss mechanism could be the cause for the low NIH shift values observed for the nitrobenzene substrate, but this is not confirmed in this experiment. The formation of phenolic products, provide evidence of an electrophilic addition reaction coupled with a shift of hydrogen or deuterium. There is a ketone intermediate common to all the reactions, due to the NIH shift values, the improbable formation of an arene oxide, and the loss of deuterium during the hydroxylation of nitrobenzene. The enzyme used has a direct effect on the site where hydroxylation occurs and the degree of the NIH shift during hydroxylation of ring substrates. These results are suggestive of a radical or carbocation formation. Figure 3 below provides a view of a reaction that involves a radical and cationic intermediate presented in article #11.
Radiolabeling and deuterium retention values can be used to determine the position of hydroxylation and help with the synthesis of some compounds. The metabolic activity of toxic chemicals and radio labeling of metabolites in vivo are examined using the hydroxylation reactions that involve the NIH shift. In article #8 the use of radiolabeled 14C and 3H-tyrosine to determine the specificity and distribution of the incorporation of tyrosine to determine if a hydroxylated species can be incorporated into tunichrome in vivo with ease in comparison to phenylalanine incorporation. Tyrosine can be incorporated into tunichrome in vivo with a higher rate constant than phenylalanine and tunichrome synthesis occurs at a lower intracellular concentration of tyrosine than phenylalanine, which its low y-intercept value provides proof of. When the 3, 5 L-tyrosine was used the 3 and 5 carbon sites were hydroxylated and is a requirement for the biosynthesis of tunichrome. The pyrogallol moiety of tunichrome retains a small percentage of the tritium label and is consistent with the NIH shift mechanism. This loss of tritium proves that tyrosine can be incorporated into tunichrome in vivo. The radiolabels are incorporated into many other tissues of A. ceratodes, which limits the usefulness of this
labeling technique because tunichrome is not the only compound labeled. Article #5 describes a synthetic technique that produces carvone labeled with stable isotopes, at a particular site to examine the oxidation mechanism of the isopropyl group of the monoterpenoid carvone. Carvonic acid was formed by oxidation at the methyl carbon of the isopropenyl group of carvone, whereas dihydrocarvonic acid was formed by oxidation at the methylene position, most probably via carvone epoxide. A nonaromatic NIH shift must occur during the subsequent reactions yielding dihydrocarvonic acid and dehydrogenation of dihydrocarvonic acid and hydrogenation of carvonic acid were observed, resulting in minor amounts of both acids owning a carboxy group of opposite origin. Uroterpenolone was found to be formed by oxidation at the methylene carbon of the isopropenyl group of carvone, and thus, most probably by hydrolysis of carvone epoxide. There are at least two different mechanisms of oxidation that must be present, because the distribution of deuterium is not consistent with one of the three possible pathways alone. Since about half of M1 carries only one deuterium, there must be the possibility of H-D exchange during metabolism. The existence of nearly 20% of mono-deuterated carvonic acid shows that the reaction between both acids has to be reversible to some extent, as from the possible pathways only nondeuterated or double-deuterated carvonic acid can be formed directly. However, labeling with 13C is necessary to prove whether the undeuterated acids stem from complete deuterium exchange or from oxidation at the methylene position which, should also result in complete loss of deuterium. The ion with $m/z$ 137, is the only one suitable for successful determination of the 13C distribution between the methyl and carboxy position. The retention of deuterium in both acids suggest that more than one oxidation method is present in this mechanism.
There are 63% of the carboxy groups contain 13C and thus stem from the methylene carbon, whereas about 37% do not contain the label and, therefore, must stem from the methyl carbon of the isopropenyl group of carvone. There is about 78% of the 13C label in M2 are still at the methylene position, whereas the remaining 22% have to be located at the carboxy group forming unlabeled ion m/z 133 in 13C-M2-ET. The distribution found in M1, shows 64% of the label was located at the carboxy group and only 36% was located at the methyl group. There is 29% D2-M1 which shows that reduction of D2-M2 without loss of deuterium is possible, since there is no other formation pathway for D2-M1. The presence of D1-M2 proves, the reverse reaction or the dehydrogenation of D2-M1, is possible. The same reaction will also generate D0-M2 from both D0-M1 or D1-M1 being labeled at the carboxy-group. Therefore, the amount of 22% M2 labeled at the carboxy group can be explained without the symmetrical-radical mechanism. The combined results of D2 and 13C labeling for M1 prove a hydride shift during the process of oxidation. If a methylene group would be oxidized to a carboxy group without a hydride shift, more than 63% of M1 would be expected not to carry any deuterium. However, only about 24% of M1 was found undeuterated and nearly 48% was found mono-deuterated which can be explained only by the NIH shift. M3 is generated by pathway 1 because the deuterium labels were found at the primary alcohol function because the usual fate of an aliphatic epoxide-equivalent is observed resulting in 1,2-diol formation after hydration. There is 23% of carboxy labeled M1 that does not contain any of the initial hydrogens from carvone, because the product of the NIH shift loses some of the hydride before oxidation because of the partial enolization. In pathway #1 oxygen addition to the methylene carbon of carvone breaks the double bond and rearrangement
results in a double bond forming a carboxy group. Oxidation of this compound results in the dihydrocarvonic acid (M1), where the OH adds to the carbonyl group. If dehydrogenation of (M1) occurs carvonic acid results with a double bond on the methylated carbon and (M3) can form from the carvone epoxide that results from the addition of oxygen to carvone. In conclusion the authors provide evidence that the partial transformation of carvone to a carvone epoxide that rearranges to give M1 can further react to give M2 by the oxidation of 10-hydroxy-carvone and M3 can be produced from the hydration of the carvone epoxide.

The addition of an alkyl and acyl group to an aromatic ring are electrophilic aromatic substitution reactions called Friedel-crafts alkylations and acylations. In the presence of aluminum chloride the mixture of benzene with an alkyl halide results in an alkyl benzene and a HX compound. This reaction involves attack of the reaction intermediate
(eg., carbocation) with the ring to form a cation intermediate that regenerates the ring. In acylation the acyl halide group in the presence of aluminum chloride results in a ketone. These substituents are moderately activating and ortho/para-directing substituents.

The ease of the NIH shift of the hydrogen and the methyl group in article #1 is consistent with an aromatic electrophilic substitution reaction in which the oxygenating intermediate forms a cation. Article #4 determines the position and the amount of deuterium when 4H and 5H-tryptophan are used. Hydroxylation occurs primarily at the 5th position and the amount of deuterium is 67% for the 5-H-tryptophan and 81% for the 4-H-tryptophan both at the 4th position. This reveals that an NIH shift occurs in the 5-H-tryptophan to the 4th carbon from the 5th carbon but no NIH shift was observed for the 4-H-tryptophan because the site of hydroxylation is on the 5th carbon of the ring. In article #3 authors probe the hydroxylation regioselectivity through the characterization of methyltryptophans and azatryptophans to examine the steric bulk and electrostatic influences these substrates have on the tryptophan hydroxylase. The evidence presented suggest that the amino acid has a single binding mode in the active site and that hydroxylation favors the 5th carbon on the ring which requires a NIH shift from the 4th carbon to the 5th carbon. The products of the reactions show that the regiospecificity of tryptophan hydroxylase is strict. Hydroxylation does not occur at the 4 or 6 carbons in response to changes in substrate topology or atomic charge. 5-hydroxymethyltryptophan and 5-hydroxy-4-methyltryptophan are the products from 5-methyltryptophan. These products establish that the hydroxylating intermediate is sufficiently potent to hydroxylate benzylic carbons and that the direction of the NIH shift in tryptophan hydroxylase is from carbon 5 to carbon 4. Hydroxylation is least efficient when the methyl group resides on the 4- or 5-
carbon of the indole ring. These results suggest that carbons 4 and 5 of the indole ring are tightly packed in the active site, in close proximity to the hydroxylating intermediate. Substituents in these positions would prevent correct binding of the indole ring and disrupt the hydroxylation reaction. The kinetic parameters in both studies provide evidence that oxygen addition to the amino acid occurs in a rate limiting step and that the initial catalytic event involves the formation of the intermediate. The evidence suggest that the amino acid has a single binding mode in the active site and that hydroxylation favors the $5^{th}$ carbon on the ring. The kinetic parameters in both studies provide evidence that oxygen addition to the amino acid occurs in a rate limiting step and that the initial catalytic event involves the formation of the hydroxylating intermediate Scheme #3 below, shows a mechanism for tyrosine hydroxylase.

The biosynthesis and biotransformation of compounds through hydroxylation is examined using biomimetic chemistry. The copper (I) reaction sites were investigated using a synthetic model to probe there activity in article #6. The dinuclear complex 5 and trinuclear copper (I) complex 6 both react with oxygen unlike the mononuclear copper (I) complexes which have very high oxidation potentials and thus little or no reactivity with
oxygen. In the dimeric copper (II) complex, the NIH shift of the ethyl group is observed and the hydroxylation reaction provides evidence of the formation of a cationic intermediate. The two complexes 5 & 6, exhibit an NIH alkyl shift where the ethyl group of the spacer migrates in a 1, 2 shift and the hydroxylation occurs at the site that the ethyl atom leaves. The NIH shift in these rearrangements provides strong evidence of a cationic intermediate but there was not an intermediate found in this study.

In article #12 the oxidative free radical mechanism of 1, 4-quinone derivatives employs a 1, 2-acyl migration (NIH shift) of the acyl group. There was proof of an imine radicals formation when the beta enamino carbonyl is oxidized by the two salts, and formation of a radical from the oxidation of the alpha-chloro-beta-ketoester adds to the double bond of the 1,4-benzoquinone. The mechanism is regioselective due to the addition of the variety of substituents to the indoles 2C position.

Nucleophilic aromatic substitution is a reaction that replaces a halogen or some other nucleophile with another nucleophile on the aromatic ring. These nucleophilic aromatic substitutions involve aryl halides that starts with dehydrohalogenation and results in a
benzyne intermediate, followed by a nucleophilic addition of an amide ion to the benzyne triple bond owing to a carbanion intermediate. A proton transfer from ammonia to the carbanion intermediate gives the product and an amide ion.

Biomimetic chemistry involves the study of the structure and function of biological systems as models for the design of materials. In article #14 the authors provide sufficient evidence that an electron rich intermediate can be oxidized causing an intramolecular transfer of the oxygen atom to the methoxyvinyl group. These intermediates act as nucleophilic oxygen transfer reagents in oxygenation and others have proved the transfer of an oxygen atom from these intermediates allows them to be used as models for some reactions catalyzed by monooxygenase enzymes. Carbonyl oxides are poor reagents for epoxidation of olefins, but the data here suggest that in some instances, the oxygen transfer process can provide an efficient route. There is evidence of the epoxidation of electron rich olefins with the intramolecular oxygen transfer from carbonyl oxide intermediates as an efficient alternative to the process. The intermolecular transfer of the oxygen atom proves to be a slow process that employs other reactions that have precedence over the actual oxygen transfer.
An aryl migration involves an aryl group that undergoes nucleophilic aromatic substitution where the site of the nucleophile or halogen on an aromatic ring is replaced by another nucleophile. In this reaction dehydrohalogenation of the ring forms a benzyne intermediate that upon addition of an amide ion forms a carbanion intermediate. Proton transfer from ammonia to the carbanion intermediate gives the substitution product along with an amide ion. In article #7 the synthesis of an iron (II) complex is used to investigate the mechanism of aryl hydroxylation reactions using non heme iron centered enzymes to provide insight into there mechanism and kinetics in these reactions. The arene hydroxylation in the iron (II) complexes involves a one-electron oxidation of the iron complex by the peroxide to an iron (III) alkylperoxo intermediate. The break down of the peroxo intermediate and the arene hydroxylation is coupled. Efficient hydroxylation of arenes observed when the iron (III) complexes with peroxide or dioxygen/ascorbic acid are used. There is evidence of an oxoiron (IV) in the arene hydroxylation reactions that result from an NIH shift. The high concentration of the copper complexes 1 and 2 produce intermolecular (lg 2:2-peroxo) Cu (II) 2, and at low concentrations the
complexes produce intramolecular copper (II) species. The hydroxylation of the 2 position of the linker with a NIH shift of the methyl group is from decomposition of the dioxygen compound, and is indicative of an electrophilic aromatic substitution that involves a carbocation intermediate. Article #7 shows how the addition of different alpha aryl substituents along with electron withdrawing functional groups at the meta position form the L2-L6 ligands. These meta substituents help in the manipulation of the electronic properties at the ortho position, while the aryl substituents allow for aromatic electrophilicity of the ring. The deshielding effects of the meta substituents on the para and ortho positions provides evidence of these findings. The para and ortho proton resonances described provide evidence that supports the notion that the ipso and other meta protons were unaffected by the meta substituents. The iron (II) complexes were made by mixing the Fe (II) (OTf) 2 and 2 methyl carbon nitrate together yeilding neutral iron (II) complexes dissolved in methyl carbon nitrate to form bis salt cations. There were three complexes obtained namely L1, L6, and L8. L1 and L6 have a geometry that is similar in comparison to the tetradentate ligands, a pseudooctohedral coordination which provides open cis sites and a pyridine with two ligands. The bond lengths are described as being in the range of (~2.18 & ~2.22) which is evidence of a high spin iron (II) state for the L1 and L8 complexes, with the exception of L6 that has a slightly higher bond length making this complex non symmetric. When the seven triflate complexes dissolved in methylchloride were examined, the light yellow solution gave poor signals with very low absorption in the 350nm-380nm region. In the organonitrile solution the iron (II) and pyridine shows strong absorbance signals at 330(+/-) 5 nm and 400(+/-) 5 nm and a small but broad weak signal at 550 nm in the ligand field. The organonitrile solutions have
better absorbance than the methylchloride solutions due to the color change. The results show that the weaker ligand fields are attracted to the iron (II) ion. This provides a practical method that can be used once the stable chromophors are developed. The L2, L3, and L4 complexes containing aryl substituents caused a blue shift, while the L5 complex was red shifted relative to the L1 chromophor. This red shift is due to a decrease in the basicity because of the methylated ligand. These chromophores in these complexes show evidence of an otho hydroxylatation of the pendant arenes with LMCT bands that are characteristic of the phenolate to iron (III) bands. There was an oxo-dimer detected in the L1 complex.

The (1, 2)-Aryl (NIH) shift in the synthesis of substituted indoles provides evidence of the development of an efficient regioselective synthesis of indole using a wide variety of organometallic reagents in article #12. This reaction proceeds with the 1, 2-aryl rearrangement followed by intramolecular condensation to form an indole. The substituents are introduced into the carbon 2 site of the indole ring and different substitution patterns on the alpha-chloro ketone. This proves that there is stringent regioselectivity in this compound. In article #13 the manganese (III) free radical reactions provide an effective method for the formation of spirolactum and the 2, 4, 7-trione. The results of this study prove that the reaction of the 2-hydroxy-1, 4-naphthoquinone with beta enamino carbonyl with (TBACN), produces the spirolactum(6) in high yields, although the combination of TBACN/CHCl3 solvent provides the best reaction conditions for the formation of the complex. Carbonyl oxide moieties have been examined for there role in the ozonolysis mechanism. These intermediates act as
nucleophilic oxygen transfer reagents in sulfoxides and olefin oxygenation. Previously, studies suggest that the transfer of an oxygen atom from these intermediates allows the carbonyl oxides to act as models for some reactions catalyzed by monooxygenases. In article #2 the purpose was to determine the retention of toxic PCB’s in the blood and tissues of the rats tested using the mechanisms of hydroxylation and methiolation. The metabolism of pentaCB and hexaCB substituted congeners show a lower formation of methylsulfonyl metabolites than the hydroxyl products. The blood of the rats retained the highest percentage of hydroxyl metabolites oppose to the other organs and tissues tested. The hydroxyl metabolites retained were formed due to a NIH shift at the 4th position chlorine substituent. Hydroxylation proceeded primarily at the meta or para position either with an arene oxide involving the NIH shift and dechlorination, or through direct oxygen insertion of a hydroxyl group. The schematic view below shows a pathway for the hydroxylation of PCB cogeners that begins with epoxidation, followed by direct oxygen insertion, dechlorination and the NIH shift.
In my research most of the participants used the NIH shift to determine the existence of a cationic intermediate. The intermediates or cationic species are reactive and could cause formation of other compounds, therefore their presence in these reactions are of significance to the mechanisms studied. There are a variety of hydroxylation reactions that involve the NIH shift and I surveyed several of these reactions to further explain the importance of this chemical shift in the hydroxylation of aromatic compounds.

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