INQUIRY & INVESTIGATION

The Exposome: Making Chemical Exposures Relevant to Biology Instruction

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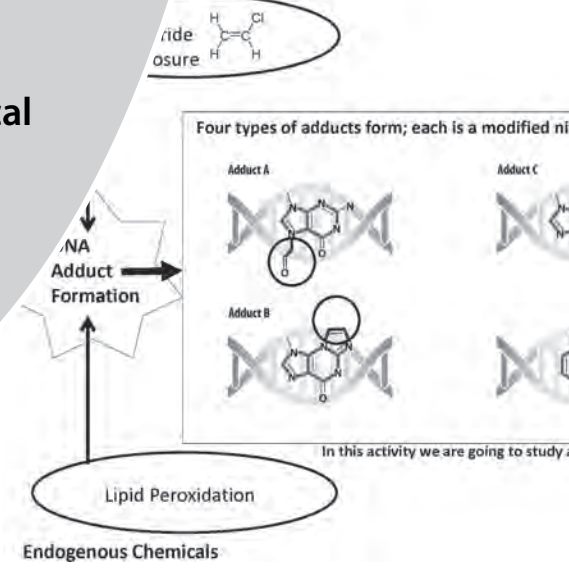

Abstract

"Investigating the Exposome: Vinyl Chloride Exposure, DNA Damage & Repair" is a data interpretation and graphing activity that provides students a relevant context in which to explore and refine what they know about DNA structure and function, mutation, DNA repair, and cancer formation. The activity utilizes published scientific data and invites students to assess the impact of exposure to vinyl chloride on DNA in an effort to introduce them to the concept of the exposome and engage them in this exciting field of scientific research.

Key Words: *biomarker; cancer; carcinogen; DNA; DNA adduct; exposome; mutation; Superfund; vinyl chloride.*

○ Introduction

Humans are exposed to many chemicals throughout their lifetime. The totality of these exposures is known as a person's *exposome*. First introduced in 2005, the concept of the exposome has fueled a body of scientific research and has tasked scientists with distinguishing between exposures to endogenous chemicals (formed inside the cells) and exogenous chemicals (from the environment) in order to assess their impact on human health (Wild, 2005; Swenberg et al., 2011). With >85,000 chemicals approved under federal law for commercial use in the United States, the topic of the exposome is relevant to everyone, given that humans are exposed to chemicals in utero and for the remainder of their lives (U.S. Environmental Protection Agency, 2017). The development of cancer (carcinogenicity) is the major human health risk associated with exposure to many toxic chemicals, including those present in our everyday lives as well as those commonly encountered at Superfund sites, the country's worst hazardous waste sites.

Here, I describe a data interpretation and graphing activity that showcases the scientific process being used to investigate mechanisms of cancer formation (carcinogenesis), with emphasis on the role of DNA damage and repair in response to exposure to cancer-causing chemicals such as vinyl chloride. Specifically, this activity requires students to construct bar graphs and compare the rates of formation and repair of three different DNA adducts that form in response to vinyl chloride exposure. Students then evaluate whether any have the potential to serve as a clinical biomarker of vinyl chloride exposure. This activity is aligned to the Advanced Placement Biology curriculum and to the *Next Generation Science Standards* (NGSS). It utilizes all three dimensions of the NGSS to introduce students to the emerging science of the human exposome through the introduction of core ideas and cross-cutting concepts and the utilization of science and engineering practices.

○ Learning Objectives

Upon completion of this activity students will be able to

- define the term *exposome*,
- distinguish between endogenous and exogenous chemical exposures,
- define a DNA adduct and describe the consequences if an adduct does not get repaired, and
- interpret authentic data to distinguish between endogenous and exogenous adducts and characterize the rate at which they are repaired.

○ Background

Cancer is a multistep disease resulting from genetic alterations to DNA, and mutation is a major mechanism for inducing such alterations. A scientist at the University of North Carolina at Chapel Hill, James Swenberg, is studying the impact of exposure to chemicals on our DNA in hopes of informing adequate risk assessment, monitoring chemical exposures, and informing regulation of chemicals

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in our environment, at hazardous waste sites and in everyday consumer products.

In addition to examining the role of exogenous chemical exposures in inducing DNA damage, Dr. Swenberg also studies DNA damage resulting from endogenous exposures to chemicals that arise mainly from oxidative stress and other intracellular processes that generate reactive chemicals. DNA damage arising from endogenous exposures can cause background mutations; thus, there are steady-state amounts of endogenous DNA damage in our cells, an estimated 40,000 damage sites (or lesions) per cell (Nakamura et al., 2014). Dr. Swenberg's working hypothesis is that background mutations are induced by endogenous mutagenic DNA damage and that exogenous exposure to a chemical can either introduce a different spectrum of DNA mutations or introduce DNA damage identical to that induced by the endogenous chemical.

Dr. Swenberg's research team has been at the forefront of developing and validating a comprehensive set of biomarkers that can be used to identify DNA damage caused by exposure to both endogenous and exogenous chemicals. A biomarker can be thought of as a chemical "fingerprint" that is generated upon exposure to a chemical. Biomarkers can be short-lived if repaired or they can linger; if not repaired, they can result in abnormal gene function and cell death and ultimately contribute to the formation of cancer. DNA adducts are one category of biomarkers. DNA adducts occur when a chemical covalently binds to DNA and can form in response to a chemical exposure, thus representing one type of DNA damage.

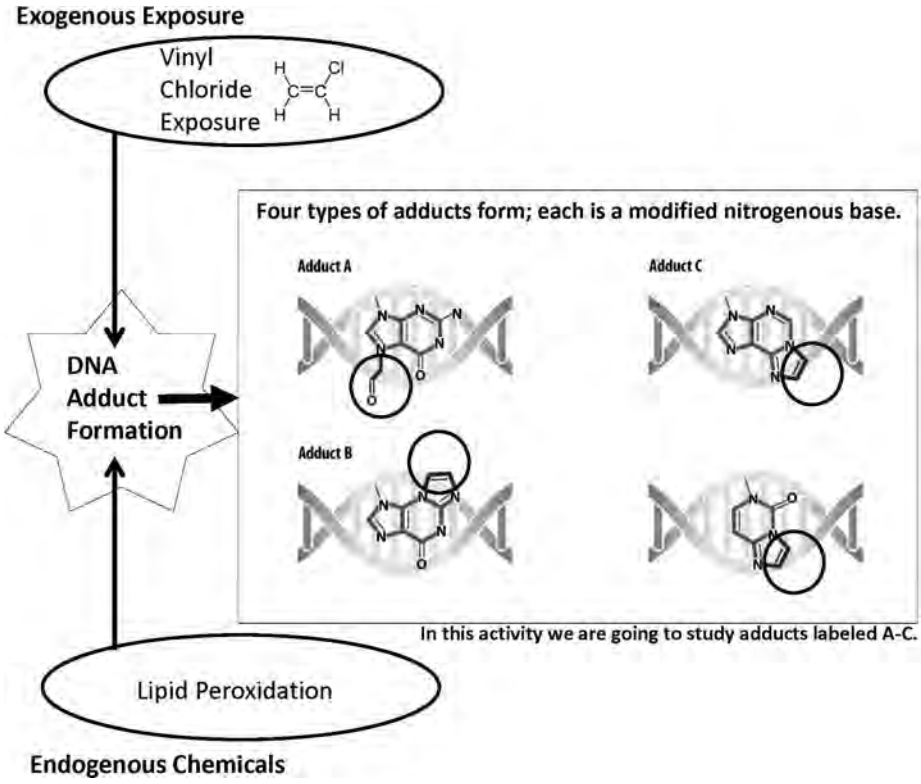

Figure 1. Four DNA adducts can result from exposure to vinyl chloride or to endogenous chemicals resulting from lipid peroxidation. Three (Adducts A–C) are the focus of this activity. Note that these are all modified nitrogenous bases (the covalent modifications are circled).

In this activity, students analyze data from Dr. Swenberg's research to study the impact of vinyl chloride exposure on the formation and repair of DNA adducts. Vinyl chloride (VC) is an industrial chemical and also a component of tobacco smoke. It is also formed as the byproduct of microbial action (specifically dechlorination) on perchloroethylene (PERC) and trichloroethylene (TCE), two chemicals commonly found at Superfund sites, recognized as the country's worst hazardous waste sites. It is worth noting that VC is not a chemical intentionally left behind at Superfund sites, but rather is the byproduct of the microbial degradation of PERC and TCE (Pottenger et al., 2014). VC is a known human carcinogen (ATSDR, 2006). Inside the body, VC is converted into chloroethylene oxide (CEO), which covalently binds to nitrogenous bases to produce four kinds of DNA adducts (Figure 1); however, research has shown that these four DNA adducts are identical to adducts induced by endogenous exposure to chemicals produced by cells as a result of lipid peroxidation and oxidative stress. In order to characterize and assess the human health risk resulting from exogenous exposure to VC, scientists must be able to distinguish between adducts resulting from exogenous exposure to VC and those that form via endogenous processes. The formation of these exogenously derived adducts is thought to be important in mediating VC's carcinogenic effects, with each adduct having different biological activity and contributing to VC's toxicity in different ways. Three of the four adducts are the focus of Dr. Swenberg's research (Table 1; Pottenger et al., 2014).

These DNA adducts are produced in different amounts, with Adduct A being the major adduct formed, comprising ~90% of adducts generated in response to VC exposure, or about 1000× greater than Adducts B and C. To distinguish between adducts produced by endogenous cellular activity and exogenous exposure to VC, scientists can use stable-isotope labeled ($^{13}C_2$)-VC and track its incorporation into DNA adducts in rodents (Figure 2). Adducts resulting from exogenous exposure to VC will have a higher mass than adducts produced by endogenous exposure. In this activity, students will analyze data and determine the extent to which endogenous and exogenous VC-induced DNA adducts form and the extent and speed of adduct repair.

○ Teacher Preparation & Materials

- Read the background information, review the activity procedure and accompanying PowerPoint (PPT) slide set, and add any additional figures/slides if desired. The Lesson Plan with

Table 1. Characterization of the three adducts that are the focus of this activity. Each adduct is a modified nitrogenous base.

DNA Adduct[a]	Abbreviation	Action	Half-life (Liver)
7-(2-oxoethyl)guanine (Adduct A)	7-OEG	Lacks miscoding properties and is removed from DNA by chemical depurination	4 days
N^2,3-ethenoguanine(Adduct B)	εG	Promutagenic activity during DNA synthesis	150 days
1,N^6-ethenodeoxyadenosine (Adduct C)	εdA	Promutagenic activity during DNA synthesis	~1 day

[a]For the purpose of this activity, the scientific names of these adducts are not important. 7-OEG will be referred to as Adduct A; εG will be referred to as Adduct B; and εdA will be referred to as Adduct C on the student worksheet.

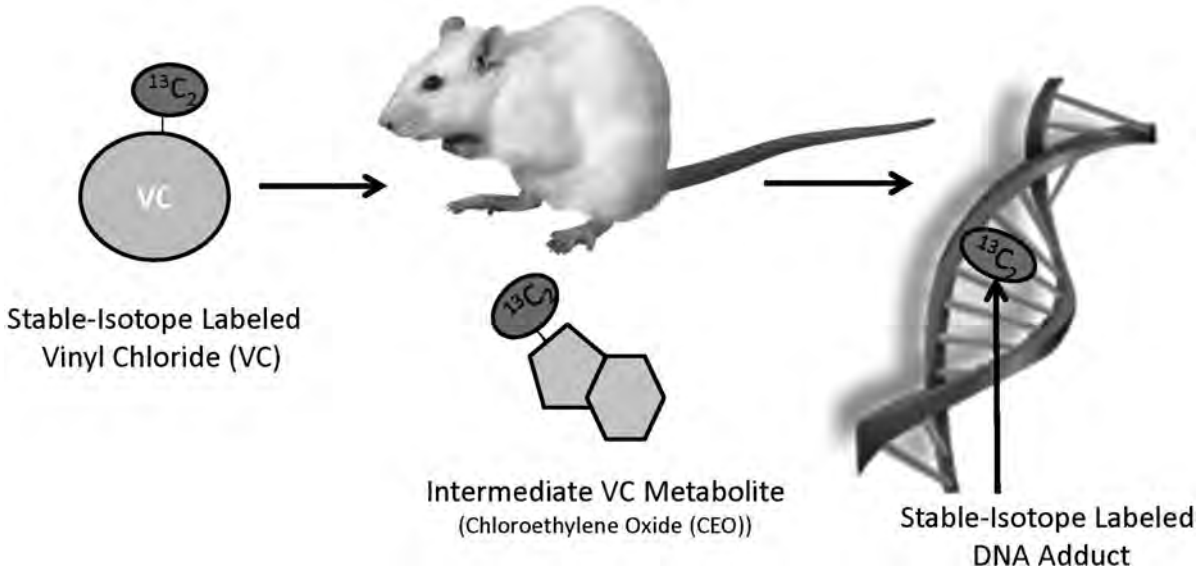

Figure 2. Scientists can use stable-isotope labeled ($^{13}C_2$)-VC and track its incorporation into DNA adducts. VC-induced adducts will have stable-isotope labels; endogenous adducts will not. In this study, the formation of VC-induced DNA adducts was examined in rat liver tissue, the primary site of VC metabolism. Students should observe that the number of endogenous DNA adducts do not significantly change over time, as would be expected from an endogenous mutagenic chemical; there are steady-state amounts of endogenous DNA damage in our cells as new adducts are formed and repaired continuously. Students will observe greater adduct formation in response to exogenous exposure to ($^{13}C_2$)-VC and a variation in the extent to which each adduct gets repaired.

worksheet and answer key is available at https://ie.unc.edu/dna-epigenetics/. The PPT slide set is available at https://ie.unc.edu/dna-epigenetics/.

- Prepare students for this activity: they should have a basic understanding of DNA structure and function and the fundamentals of gene expression.
- Make copies of the double-sided student worksheet (see Lesson Plan and Appendix), one per student.

○ Implementation

(1) Prompt students to consider that completing the sequencing of the human genome in 2003 led to a revolution in other "-omics" sciences and technologies to better understand the interactions that take place between our genes, our proteins, and our environment and to elucidate causes of human disease. Our environment includes tens of thousands of chemicals that we are exposed to throughout our lifetime, starting in utero; thus, an investigation of gene–environment interactions must include chemical exposures.

(2) Introduce students to the concept of the exposome (see Teacher PPT slide 2), which was first introduced in 2005 (Wild, 2005) and refers to an individual's lifetime exposure to chemicals from the environment coupled with exposure to chemicals formed inside our cells as a consequence of metabolic processes. Your students may have never considered that their very own cells produce chemicals, some of which are toxic.

(3) Distinguish between endogenous and exogenous exposure to chemicals and discuss the relevance in relation to chemical exposure (improved risk assessment, cancer, etc.). When evaluating the toxicity of chemicals in our environment, scientists need to be aware if these toxic effects can

Table 2. Relative amounts of endogenous and exogenous DNA adducts in liver DNA from rats exposed to [$^{13}C_2$]-VC (1100 ppm, 6 hr/day, 5 days). Liquid chromatography tandem mass spectrometry (LC-MS/MS) was used to quantify adduct formation in liver tissue. The number of adducts for every 10^5 or 10^8 guanine (G) or 10^8 adenine (dA) nucleosides are reported as the mean and standard error (+/-) of the mean. Standard error of the mean is calculated to describe the variation in each data set with smaller standard error values denoting less variation from the mean. In general, standard error bars that do not overlap suggest that the difference between two mean values may be statistically significant and therefore unlikely the result of chance, but one must perform a statistical test to draw a conclusion.

	Adduct A		Adduct B		Adduct C	
	[$^{12}C_2$]-7OEG/ 10^5 Gua	[$^{13}C_2$]-7OEG/ 10^5 Gua	[$^{12}C_2$]-N^2,3-εG/ 10^8 Gua	[$^{13}C_2$]-N^2, 3-εG/10^8 Gua	[$^{12}C_2$]-1N^6-εdA/10^8 dA	[$^{13}C_2$]-1N^6-εdA/10^8 dA
	Endogenous	***Exogenous***	***Endogenous***	***Exogenous***	***Endogenous***	***Exogenous***
Two Hours Post Exposure	0.2 ± 0.1	10.4 ± 2.3	4.1 ± 2.8	18.9 ± 4.9	4.9 ± 0.6	5.1 ± 0.6
2 Weeks Post Exposure	0.1 ± 0.03	0.4 ± 0.3	3.7 ± 3.1	14.2 ± 4.2	8.6 ± 0.9	Not detected
4 Weeks Post Exposure	0.2 ± 0.04	0.1 ± 0.06	3.1 ± 1.0	16.9 ± 1.6	6.2 ± 1.3	Not detected
8 Weeks Post Exposure	0.2 ± 0.07	Not detected	3.7 ± 1.5	13.2 ± 2.5	4.1 ± 0.5	Not detected

also arise through normal cellular metabolism, to get a more accurate sense of the extent to which exposure occurs and to better understand the implications for human health.

(4) Introduce students to the research of James Swenberg, a leading toxicologist investigating DNA damage caused by exposure to both endogenous and exogenous chemicals such as vinyl chloride and formaldehyde. A three-minute video summarizing his research can be found at https://www.youtube.com/watch?v=O-2T6eTE_bM.

(5) Introduce (or review) the mechanisms of DNA damage, being sure to include a description of DNA adducts. DNA adducts form when a chemical covalently binds to the DNA molecule; in this case it is nitrogenous bases that become altered. DNA adducts are being studied as potential biomarkers for chemical exposure.

(6) Tell students that they are going to learn about one chemical that is a known human carcinogen, vinyl chloride (VC). In the case of VC, it is converted into chloroethylene oxide (CEO), which covalently binds to DNA to produce four kinds of DNA adducts (see Teacher PPT slide 7). Tell them these four adducts can also occur inside cells under normal metabolic conditions in the absence of VC exposure.

(7) Introduce students to the challenge that arises when a chemical like VC induces the same kind of DNA adducts that are produced as a consequence of normal metabolic processes. Ask students how a scientist might go about distinguishing between adducts arising from endogenous and exogenous exposures and discuss why this is important information. In this case, the scientists used stable-isotope labeled ($^{13}C_2$)-VC to distinguish between endogenously derived adducts and adducts arising from exogenous exposure to VC. DNA adducts resulting from exogenous exposure to ($^{13}C_2$)-VC will have a higher mass than adducts produced by exposure to endogenous chemicals. Students will examine data (see Table 2 or the student worksheet in the Appendix) arising from one such experiment and characterize the rate at which these adducts are repaired.

(8) Describe how scientists used stable-isotope labeled ($^{13}C_2$)-VC and tracked its incorporation into DNA adducts in male Sprague-Dawley rats to distinguish between endogenous and exogenous adducts (see Teacher PPT slides 8 and 9). (Note: Sprague-Dawley rats are commonly used in biomedical research; therefore, this study represents an opportunity to discuss the use of model organisms/tissues in research along with the benefits and limitations of extrapolating experimental results from animal studies to humans.) Scientists exposed rats to air containing 1100 ppm ($^{13}C_2$)-VC for 6 hours/day for 5 days and then measured adduct formation in liver tissue, the primary site of VC metabolism. This concentration of VC is representative of occupational exposures in the PVC industry prior to 1974 and is associated with VC-induced angiosarcomas of the liver. For reference, OSHA's current occupational exposure level for VC is 1 ppm (ATSDR, 2006).

(9) Distribute copies of the student worksheet (Appendix) and ask students to complete questions 1–4 either individually or with a partner. Review student responses to each question as a class before proceeding.

(10) Next, tell students they will complete Part II of their worksheet. Orient students to the data table in Part II before

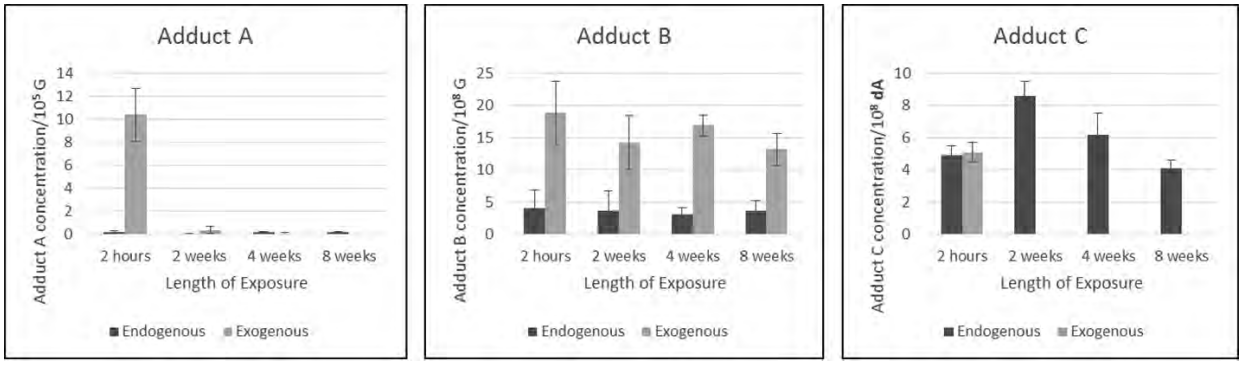

Figure 3. Completed bar graphs help students visualize experimental data (see Teacher PPT slide 13).

instructing them to construct graphs for each adduct (see Teacher PPT slide 10). Alternatively, you could divide the class into small groups and assign each group one adduct to graph; students could then share their graph with the rest of the class so that all three graphs can be compared.

(11) Students will need to observe and consider all three graphs (Figure 3) in order to complete questions in Part II.

(12) Review the answers to the questions in Part II as a class; an answer key is also available in the online version of the activity. Collectively, these three adducts can be used to illustrate to students the marked differences that exist in how a cell addresses DNA damage:

(a) The short half-life (4 days) of Adduct A is due in part to this being an unstable adduct.

(b) The long half-life (150 days) of Adduct B is thought to be due not to active DNA repair but rather to loss due to cell death and "dilution" due to cell division.

(c) The short half-life (~1 day) of Adduct C is thought to be due to the fact that there are two DNA repair pathways that can target and repair this adduct. This built-in redundancy in the DNA repair mechanism for this particular adduct means that it can be repaired very quickly. The short half-life means that much shorter time intervals are called for in any study design intended to adequately assess DNA repair; the post-exposure times (2, 4, and 8 weeks) in the featured study were too long in the case of this particular adduct.

(13) Conclude this activity by discussing the possible consequences of a DNA adduct not being repaired. A DNA adduct that is not repaired can result in the insertion of an incorrect base (base-pair substitution) in the opposite DNA strand during DNA replication or in its complementary RNA strand during transcription (see Teacher PPT slides 15 and 16). Understanding the consequences of an unrepaired DNA adduct implies that a student has a good grasp of DNA structure and function. For example, in the case of Adduct B (εG), previous research has shown that a base pair substitution occurs ~13% of the time, which represents a high mutation rate (Pottenger et al., 2014).

(14) Students may be interested to learn that in addition to VC, ethylene oxide and formaldehyde also induce exogenous DNA adducts that are chemically identical to endogenously formed DNA adducts.

(15) Remind students that knowledge gained about DNA adduct formation and repair from studies such as these on VC can be used to understand the mechanisms by which our cells respond to chemicals in the environment. Emphasize that in addition to interacting with DNA, chemicals present in our food, water, and air (and their metabolites) can interact with other macromolecules (e.g., proteins) in the cell to impact gene expression (through epigenetic mechanisms) or metabolism. Studying the impact of exposure to chemicals on our DNA informs risk assessment and the regulation of chemicals in our environment. Acknowledge that scientists interested in understanding the human exposome are turning their attention to investigating the impact of low doses of chemical exposures and of combinations of chemicals on human health. These lines of inquiry are being facilitated by advances in technology and represent an exciting area of research in the field of exposomics.

○ Assessment

Students can complete the worksheet as a formal assessment and/or they can provide a written summary of the consequences of an unrepaired DNA adduct on gene expression. A key for the worksheet and graphing activity is included in the Lesson Plan available at https://ie.unc.edu/dna-epigenetics/.

○ Opportunities for Extension

To extend this activity, invite students to conduct independent research to determine whether there are other chemicals that induce DNA adducts (some chemicals that induce adduct formation include polycyclic aromatic hydrocarbons, N-nitrosamines, and aflatoxins). Students could investigate the mechanisms by which other cancer-causing chemicals damage DNA and the extent to which biomarkers are being utilized to identify individuals that have been exposed to a particular chemical.

If you choose to do so, you could delve into specific DNA repair mechanisms. Prokaryotic and eukaryotic cells have built-in DNA repair mechanisms that include nucleotide excision repair (NER), base excision repair (BER), and mismatch repair (MMR).

○ Acknowledgements

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Appendix: Student Worksheet

Investigating the Exposome: Vinyl Chloride Exposure, DNA Damage & Repair

Part I.

Vinyl chloride (VC) is an industrial chemical, a component of tobacco smoke, and a byproduct of the microbial breakdown of perchloroethylene (PERC) and trichlorethylene (TCE) occurring at hazardous waste sites. VC is a known human carcinogen, and inside the body it is converted into chloroethylene oxide (CEO), which covalently binds to DNA to produce four DNA adducts (three of these adducts will be investigated during this activity; since their names are not important for this activity, they will be referred to as Adducts A, B, and C).

1. Define the term **exposome**.

2. Distinguish between an *endogenous* chemical exposure and an *exogenous* chemical exposure.

3. In your own words, define **DNA adduct**. Why is a DNA adduct considered a form of DNA damage?

4. Research has shown that the DNA adducts produced upon exogenous exposure to VC are identical to those produced by endogenous exposure to chemicals generated through normal cellular metabolism. Since the adducts that form upon exposure to VC are identical to adducts that form endogenously, scientists have to have some way to distinguish between the two in order to better characterize and assess human health risk resulting from exogenous exposure to VC. Propose how a scientist might do this to determine the impact of exogenous exposure of VC on DNA adduct formation.

Part II.

Use the data on the back of this sheet to construct bar graphs for Adducts A, B, and C. Once you have completed <u>each</u> graph, complete the questions below.

5. Observe all three graphs. What conclusions can you draw about endogenous adducts?

6. What conclusions can you draw about adducts induced by exogenous exposure (e.g., inhaled) VC?

7. What happens to the concentration of adducts induced by exogenous exposure to VC over time? Why?

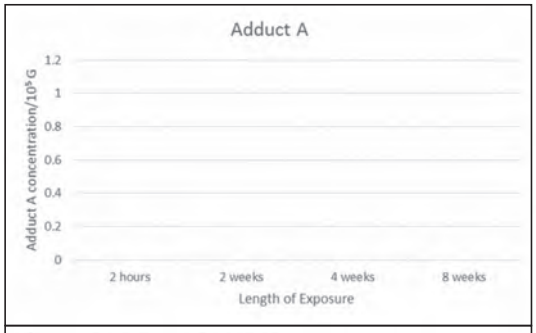
8. Which adduct (A, B, or C) appears to be removed more quickly? How do you know this?

9. It turns out that Adduct B is mutagenic, causing a base pair substitution in the complementary strand of DNA during replication or in RNA during transcription. Describe the consequences that might result from a base pair substitution occurring in DNA and RNA.

10. Which adduct (A, B, or C) would be the best biomarker to look for if VC exposure is suspected? Explain your answer.

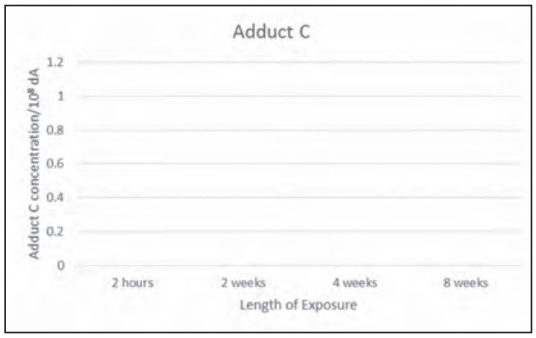
To distinguish between endogenous and exogenous adducts, scientists can use stable-isotope labeled ($^{13}C_2$)-VC and track its incorporation into DNA adducts in rodents. Scientists exposed rats to air containing 1100 ppm ($^{13}C_2$)-VC for 6 hours/day for 5 days (very high exposure!) and then measured adduct formation in liver tissue, the primary site of VC metabolism. DNA adducts resulting from exogenous exposure to ($^{13}C_2$)-VC will have a higher mass than adducts produced by exposure to endogenous chemicals. The number of adducts for every 10^5 or 10^8 guanine (G) or 10^8 adenine (dA) nucleosides is reported as mean ± standard error in the table below. Interpret the data to determine the extent to which endogenous and exogenous DNA adducts form and the extent and speed to which adducts get repaired. **For each adduct, construct a corresponding bar graph using the data points provided.** Remember to graph the error bars for each data point. Standard error of the mean is calculated to describe the variation in each data set, with smaller standard error values denoting less variation from the mean.

Time Post-exposure	Adduct A Concentration		Adduct B Concentration		Adduct C Concentration	
	Adduct A /10^5 G	**($^{13}C_2$) Adduct A /10^5 G**	**Adduct B /10^8 G**	**($^{13}C_2$) Adduct B /10^8 G**	**Adduct C /10^8 dA**	**($^{13}C_2$) Adduct C /10^8 dA**
	Endogenous	***Exogenous***	***Endogenous***	***Exogenous***	***Endogenous***	***Exogenous***
2 hours	0.2 ± 0.10	10.4 ± 2.30	4.1 ± 2.8	18.9 ± 4.9	4.9 ± 0.6	5.1 ± 0.6
2 weeks	0.1 ± 0.03	0.4 ± 0.30	3.7 ± 3.1	14.2 ± 4.2	8.6 ± 0.9	Not detected
4 weeks	0.2 ± 0.04	0.1 ± 0.06	3.1 ± 1.0	16.9 ± 1.6	6.2 ± 1.3	Not detected
8 weeks	0.2 ± 0.07	Not detected	3.7 ± 1.5	13.2 ± 2.5	4.1 ± 0.5	Not detected



Conclusions about Adduct A:

Conclusions about Adduct B:



Conclusions about Adduct C: