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

A data interpretation activity for students

Humans are exposed to many chemicals throughout their lifetime. The combination of lifetime exposure to chemicals from the environment (exogenous exposure), coupled with exposure to chemicals formed inside of our cells as a consequence of metabolic processes is known as the **exposome**. This activity introduces students to the concept of **endogenous exposure** to chemicals formed inside of our cells as a consequence of metabolic processes and the challenge that scientists face when seeking to distinguish between endogenous and exogenous exposures when it comes to assessing the impact on human health. This activity features the research of one scientist who is funded by UNC-Chapel Hill's Superfund Research Program to investigate the mechanisms of cancer formation (carcinogenesis), with emphasis on the role of DNA damage and repair in response to exogenous exposure to cancer causing chemicals.

Curriculum Alignment

Advanced Placement Biology

Big Idea 2: Biological systems utilize free energy and molecular building blocks to grow, to reproduce, and to maintain dynamic homeostasis.

Enduring understanding 2.D: Growth and dynamic homeostasis of a biological system are influenced by changes in the system's environment.

Essential knowledge 2.D.1: All biological systems from cells to organisms to populations, communities and ecosystems are affected by complex biotic and abiotic interactions involving exchange of matter and free energy.

Essential knowledge 2.D.3: Biological systems are affected by disruptions to their dynamic homeostasis.

Enduring understanding 2.E: Many biological processes involved in growth, reproduction, and dynamic homeostasis include temporal regulation and coordination.

Essential knowledge 2.E.1: Timing and coordination of specific events are necessary for the normal development of an organism, and these events are regulated by a variety of mechanisms.

Essential knowledge 2.E.2: Timing and coordination of physiological events are regulated by multiple mechanisms.

Big Idea 3: Living systems store, retrieve, transmit, and respond to information essential to life processes.

Enduring understanding 3.A: Heritable information provides for continuity of life.

Essential knowledge 3.A.4: The inheritance pattern of many traits cannot be explained by simple Mendelian genetics.

Enduring understanding 3.B: Expression of genetic information involves cellular and molecular mechanisms.

Essential knowledge 3.B.1: Gene regulation results in differential gene expression, leading to cell specialization.

Big Idea 4: Biological systems interact, and these systems and their interactions possess complex properties.

Enduring understanding 4.C: Naturally occurring diversity among and between components within biological systems affects interactions with the environment.

Essential knowledge 4.C.2: Environmental factors influence the expression of the genotype in an organism.

Next Generation Science Standards (NGSS)

Scientific and Engineering Practices

Asking questions and defining problems
Analyzing and interpreting data
Developing and using models
Constructing explanations
Obtaining, evaluating, and communicating information

Crosscutting Concepts

Patterns
Cause and effect: mechanism and explanation
Scale, proportion, and quantity
Systems and system models
Structure and Function
Stability and change

Disciplinary Core Ideas in Life Science

LS1: From Molecules to Organisms: Structures and Processes
LS3: Heredity: Inheritance and Variation of Traits

Learning Objectives

Upon completion of this lesson 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 prior to DNA replication or transcription.
- Interpret scientific data to distinguish between endogenous and exogenous induced adducts and characterize the rate at which they are repaired.

Activity Description

This brief data interpretation activity focuses on the research of one scientist who is studying the mechanisms of cancer formation (carcinogenesis), with emphasis on the role of DNA damage and repair in response to exposure to cancer causing chemicals. Specifically, students will learn about DNA adducts and interpret data to distinguish between adducts derived from endogenous chemical exposure and adducts derived from exogenous exposure to a chemical (vinyl chloride). Students will practice constructing bar graphs and will include error bars in their graphing analysis.

Teacher Preparation

- Students should have a basic understanding of DNA structure and function prior to introducing this activity.
- Students should understand the consequences of a mutation in DNA in terms of gene expression.
- Read background information, review activity procedure and accompanying PPT slide set, add any additional figures and/or slides if desired.
- Make copies of the double-sided student worksheet, one per student.

Background

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.

Cancer is a multi-step disease resulting from genetic alterations to DNA, with **mutation** being a major mechanism for inducing alterations to DNA. UNC-Chapel Hill scientist James Swenberg, DVM, PhD, 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 in our environment, at Superfund sites and in everyday consumer products.

In addition to seeking to understand the role of chemicals from our *external environment* (**exogenous exposure**) in the damage of DNA, Dr. Swenberg also examines DNA damage resulting from **endogenous exposure** to chemicals that arises mainly from oxidative stress and other intracellular processes that generate reactive chemicals. DNA damage arising from endogenous exposure to chemicals can cause background mutations; thus, there are steady-state amounts of endogenous DNA damage in our cells, an estimated 40,000 lesions per cell! 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 it might 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, and if not repaired, could result in abnormal gene function, cell death and ultimately contribute to the formation of cancer. **DNA adducts** are one form of DNA damage that results when a chemical *covalently binds* to DNA**.** DNA adducts are one form of biomarker.

A three minute video summarizing Dr. Swenberg's work can be found at: https://sph.unc.edu/superfund-pages/research-projects/biomedical/biomarkers-of-exposure-versus-effect-improving-the-scientific-basis-for-risk-assessment/

In this activity, students will analyze data resulting from Dr. Swenberg's work 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 by-product of microbial action (specifically dechlorination) on perchloroethylene (PERC) and trichlorethylene (TCE), two chemicals commonly found at Superfund sites. It is worth noting that VC is not a chemical intentionally left behind at Superfund sites, but rather is the by-product of the microbial degradation of TCE and PERC; for this reason VC is present in low concentrations at Superfund sites and its cleanup is superseded by the clean-up of its toxic precursors TCE and PERC. VC is a known human carcinogen. Inside the body it is converted into chloroethylene oxide (CEO) which covalently binds to DNA to produce four kinds of DNA adducts (three of them are the focus of Dr. Swenberg's research and are described in the table below). 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. Therefore **scientists must have some way to distinguish between the two in order to better characterize and assess human health risk resulting from exogenous exposure to VC.** The formation of these adducts is thought to be important in mediating vinyl chloride's carcinogenic effects, with each adduct having different biological activity and contributing to VC's toxicity in different ways.

DNA Adduct*	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)	**ƐG**	Promutagenic activity during DNA synthesis	150 days
1,N^6-ethenodeoxyadenosine (Adduct C)	**ƐdA**	Promutagenic activity during DNA synthesis	~1day

Table 1. Characterization of the three adducts that are the focus of this activity; each adduct is a modified nitrogenous base. For the purpose of this activity, the scientific names of these adducts aren't important. 7-OEG will be referred to as Adduct A; ƐG will be referred to as Adduct B; and ƐdA will be referred to as Adduct C on the student worksheet.

These DNA adducts are produced in different amounts, with Adduct A being the major adduct formed, comprising approximately 90% of adducts generated in response to VC exposure (Adduct A concentration is about 1,000 times higher than Adducts B and C). To distinguish between adducts produced by endogenous cellular activity and exogenous exposure to vinyl chloride, scientists can use **stable isotope labeled ($^{13}C_2$)-VC** and track its incorporation into DNA adducts in rodents. 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 to which adducts get repaired.**

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 stable isotope labeled VC and a variation in the extent to which each adduct gets repaired.

- The **short half-life (4 days) of Adduct A** (7-OEG) is due in part to this being an unstable adduct.
- The **long-half life (150 days) of Adduct B** (εG) is not thought to be due to active DNA repair but rather loss due to cell death and "dilution" due to cell division.
- The **short half-life (~1 day) of Adduct C** (ƐdA) 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 (2,4,8 wks) in the featured study were too long in the case of this particular adduct.

Collectively, these three adducts can be used to illustrate to students the marked differences that exist in how a cell addresses DNA damage.

You could 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. For example, in the case of Adduct B (εG), previous research has shown that a base pair substitution occurs approximately 13% of the time which represents a high mutation rate.

If you choose to do so, you could delve into the 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).

Activity Procedure

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)*. The concept of the exposome was first introduced in 2005 (see *Resources* section) and refers to an individual's *lifetime exposure* to chemicals from the environment coupled with exposure to chemicals formed inside of 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 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. Tell the students that they are going to learn about one chemical that is a known human carcinogen, vinyl chloride (see *Teacher PPT slide 3)*.

5. Introduce students to the research of Jim Swenberg, DVM, PhD, at UNC-Chapel Hill (see *Teacher PPT slide 4)*. Dr. Swenberg is 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 Dr. Swenberg's work can be found at: https://sph.unc.edu/superfund-pages/research-projects/biomedical/biomarkers-of-exposure-versus-effect-improving-the-scientific-basis-for-risk-assessment/

6. Introduce (or review) the mechanisms of DNA damage, being sure to include a description of DNA adducts (see *Teacher PPT slide 5).* DNA adducts form when a chemical covalently binds to the DNA molecule, in this case, nitrogenous bases. DNA adducts are being studied as biomarkers for chemical exposure.

7. Inside our cells vinyl chloride is converted into chloroethylene oxide (CEO) which covalently binds to DNA to produce four kinds of DNA adducts; these four adducts are modified nitrogenous (see *Teacher PPT slide 7).* Tell students these four adducts can also occur inside cells under normal metabolic conditions in the absence of VC exposure!

8. Introduce students to the challenge that arises when a chemical like VC induces the same kinds 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, scientists used stable isotope labeled ($^{13}C_2$)-VC to distinguish between endogenously derived adducts and adducts arising from exogenous exposure to VC (see *Teacher PPT slides 8-9).* DNA adducts resulting from exogenous exposure to ($^{13}C_2$)-VC will have a higher mass than adducts produced by exposure to endogenous chemicals.

9. 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 were associated with VC-induced angiosarcomas of the liver. For reference, OSHA's current occupational exposure level for VC is 1 ppm (ATSDR, 2006).

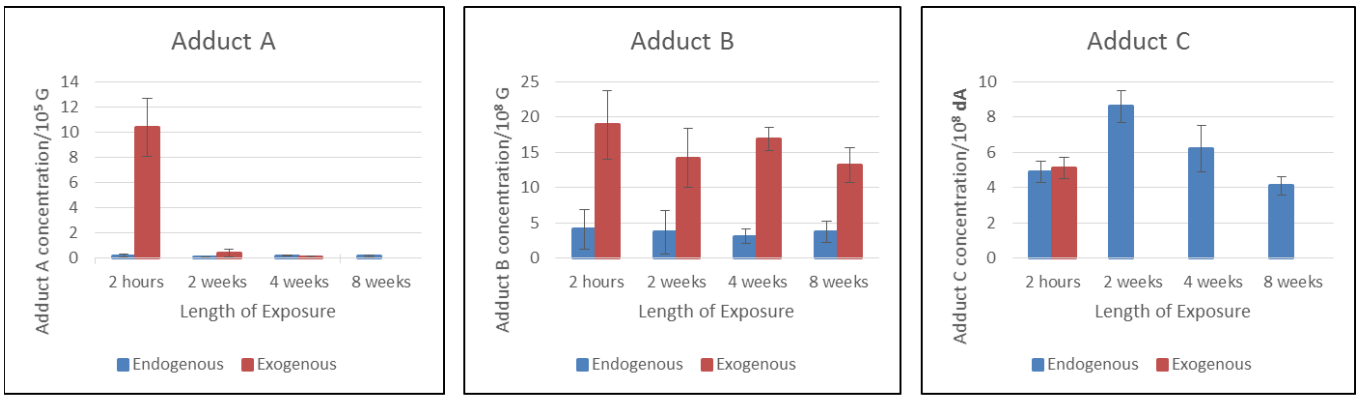
10. Distribute copies of the student worksheet and ask students to complete questions 1-4 either individually or with a partner and review student responses to each question as a class before proceeding.

11. Next, tell students they will complete Part II of their worksheet by graphing and analyzing data arising from three experiments that utilized the same study design to characterize the rate at which these adducts are repaired (See *Resources* section for research citations; 7-OEG: Mutlu, 2012; εG: Mutlu, 2010; εdA: Gao, unpublished). **Orient students to the data table in part II before having them construct bar graphs for Adducts A, B and C** (see Table 2 below and *Teacher PPT slides 10-11).*

	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

Table 2. Relative amounts of endogenous and exogenous DNA adducts in liver DNA from rats exposed to [13C2]-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.

12. Students will need to observe all three graphs in order to complete questions 5-9 in Part II. Sample graphs are provided below and in the *Teacher PPT slide 13*. Students could be asked to construct all three graphs or you could assign one adduct to each student or student group; students could then share their graph with the rest of the class so that all three graphs can be compared.



Short half-life
Unstable adduct

Long half-life
Not repaired

Short half-life
Quick repair

Figure 1. Completed bar graphs help students visualize experimental data (see *Teacher PPT slide 13*).

13. Review the answers to the questions in Part II as a class; an answer key is available on page 8.
Collectively, these three adducts can be used to illustrate to students the marked differences that exist in how a cell addresses DNA damage:
    - The short half-life (4 days) of Adduct A is due in part to this being an unstable adduct.
    - The long half-life (150 days) of Adduct B is not thought to be due to active DNA repair but rather loss due to cell death and "dilution" due to cell division.
    - 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 (2,4,8 wks) in the featured study were too long in the case of this particular adduct.

14. 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 (ƐG), previous research has shown that a base pair substitution occurs approximately 13% of the time which represents a high mutation rate (Pottenger et al., 2014).

15. 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.

16. Remind students that knowledge gained about DNA adduct formation and repair from studies such as these on vinyl chloride 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 exposure to low doses of chemicals and to 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.

Opportunities for Extension

- To extend this activity invite students to conduct independent research to determine if there are other chemicals that induce DNA adducts (some chemicals that induce adduct formation include: polycyclic aromatic hydrocarbons (PAHs), 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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Resources

Agency for Toxic Substance Disease Registry (ATSDR). (2006). Toxicological Profile for Vinyl Chloride. Available online at: https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=282&tid=51

Mutlu, E., Collins, L., Stout, M., Upton, P., Dave, L., Winsett, D., Hatch, G., Evansky, P., Swenberg, J. (2010). Development and application of an LC-MS/MS method for the detection of the vinyl chloride-induced DNA adduct $N_{(2)}$,3-ethenoguanine in tissues of adult and weanling rats following exposure to [$(^{13})C_{(2)}$]-VC. *Chem Res Toxic* 23(9): 1485-1491.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3104734/

Mutlu, E., Jeong, Y., Collins, L., Ham, A., Upton, P., Winsett, D., Hatch, G., and Swenberg, J. (2012). A new LC-MS/MS method for the quantification of endogenous and vinyl chloride-induced 7-(2-Oxoethyl) guanine in Sprague-Dawley rats. *Chem Res Toxicol* 20; 25(2):391-9. Pub 2012 Jan 24.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3288741/

Nakamura, J., Mutlu, E., Sharma, V., Collins, L., Bodnar, W., Yu, R., Lai, Y., Moeller, B., Lu, K., and Swenberg, J. (2014). The endogenous exposome. *DNA Repair*, 19, 3–13.
http://www.sciencedirect.com/science/article/pii/S1568786414001025

Pottenger, L., Andrews, L., Bachman, A., Boogaard, P., Cadet, J., Embry, M., Farmer, P., Himmelstein, M., Jarabek, A., Martin, E., Mauthe, R., Persaud, R., Preston, R., Schoeny, R., Skare, J., Swenberg, J., Williams, G., Zeiger, E., Zhang, F. & Kim, J. (2014). An organizational approach for the assessment of DNA adduct data in risk assessment: case studies for aflatoxin B1, tamoxifen and vinyl chloride. Crit Rev Toxicol. 44(4), 348-91.
http://www.ncbi.nlm.nih.gov/pubmed/24494825

US EPA. (2017). Toxic Substances Control Act (TSCA) Chemical Substance Inventory. Available online at:
https://www.epa.gov/tsca-inventory

The Exposome

C.P. Wild. (2015). Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiol. Biomarkers Prev.*, 14, 1847–1850
http://cebp.aacrjournals.org/content/14/8/1847.full.pdf+html

P. Liory, S. Rappaport. (2011). Exposure science and the exposome: an opportunity for coherence in the environmental health sciences. *Environ. Health Perspect.*, 119, A466–A467.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3226514/pdf/ehp.1104387.pdf

C.P. Wild. (2012). The exposome: from concept to utility. *Int. J. Epidemiol.*, 41, 24–32.
http://ije.oxfordjournals.org/content/early/2012/01/30/ije.dyr236.full.pdf+html

Investigating the Human Exposome
http://www.epa.gov/heasd/exposome.html

The Human Exposome project
http://humanexposomeproject.com/

Additional resources

Three- minute video about Dr. Swenberg's research
https://sph.unc.edu/superfund-pages/research-projects/biomedical/biomarkers-of-exposure-versus-effect-improving-the-scientific-basis-for-risk-assessment/

Agency for Toxic Substance Disease Registry (ATSDR). (2006). Vinyl Chloride – ToxFAQs. http://www.atsdr.cdc.gov/toxfaqs/tfacts20.pdf

FIGURE 2 | Generation of altered gene products
From the article: *Chemical-induced DNA damage and human cancer risk*
http://www.nature.com/nrc/journal/v4/n8/fig_tab/nrc1410_F2.html

Investigating the Exposome: Vinyl Chloride, DNA Damage & Repair Name: *Answer key*

Part I.

Vinyl chloride (VC) is an industrial chemical and it is also a component of tobacco smoke and it is the by-product 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 and 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**. *The combination of lifetime exposure to chemicals from the environment coupled with exposure to chemicals formed inside of our cells as a consequence of metabolic processes.*

2. Distinguish between an endogenous chemical exposure and an exogenous chemical exposure. *People can be exposed to chemicals from their external environment (exogenous) or from inside (endogenous) their bodies as a result of metabolic activity.*

3. In your own words, define **DNA adduct**. Why is a DNA adduct considered a form of DNA damage? *DNA adducts form when a chemical covalently binds to DNA. Because it covalently binds to DNA it can alter the three dimensional structure and impair DNA function and/or lead to mutation.*

4. Research has shown that the DNA adducts produced upon exogenous exposure to vinyl chloride 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 do determine the impact of exogenous exposure of VC on DNA adduct formation. *Answers will vary. Some students may come up with the idea to somehow "tag" exogenous chemicals and track their fate inside an organism – see which tags end up as adducts.*

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? *The level of endogenous adducts do not change significantly over time. Steady-state concentration.*

6. What conclusions can you draw about adducts induced by exogenous exposure (e.g., inhaled) vinyl chloride? *Exposure to exogenous VC resulted in adduct formation that exceeded the normal, steady-state concentration.*

7. What happens to the concentration of adducts induced by exogenous exposure to VC over time? Why? *Concentration decreases over time as adducts get repaired, or are lost due to cell death and cell replication.*

8. Which adduct (A, B or C) appears to be removed more quickly? How do you know this? *Adduct C followed by Adduct A. The levels of Adduct C are not detectable at 2 wks post-exposure and are significantly reduced for Adduct A, while they are still high for Adduct B.*

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. *If an adduct is not repaired this will lead to a mutation in the DNA during DNA replication. In the case of RNA, the wrong base could be inserted during transcription and depending on the location of this "mistake' translation might result in no protein product, an amino acid substitution, or a protein that is too short or too long, or it may not affect the final product at all.*

10. Which adduct (A, B, or C) would be the best biomarker to look for if VC exposure is suspected? Explain your answer. *Adduct B would be the best biomarker since it has the longest half-life.*

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

Part I.

Vinyl chloride (VC) is an industrial chemical and it is also a component of tobacco smoke and it is the by-product 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 and 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 vinyl chloride 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 do determine the impact of exogenous exposure of VC on DNA adduct formation.

Part II. Analyzing and interpreting data

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) vinyl chloride?

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

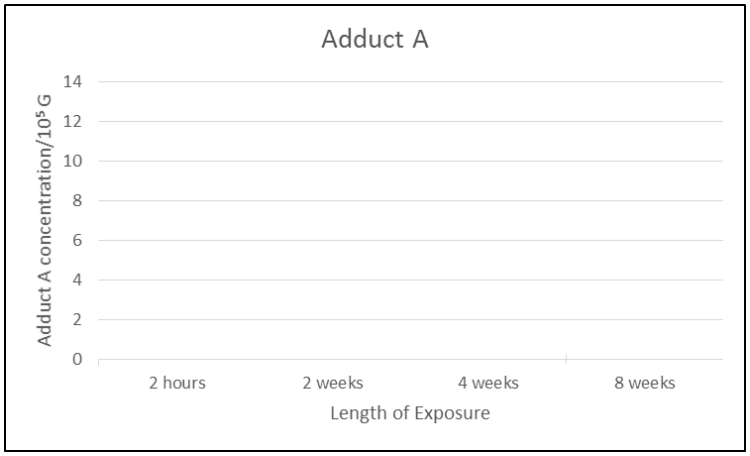
8. Which adduct (A, B or C) appears to be removed more quickly? How do you know this?

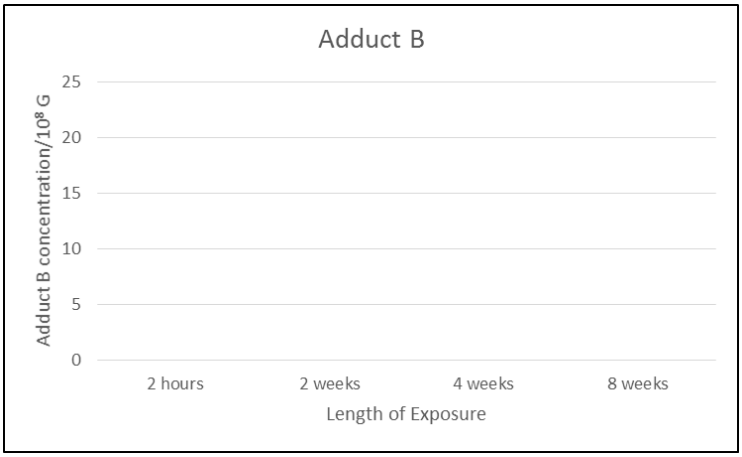
9. It turns out that Adduct B is mutagenic, causing a base pair substitution in the complementary strand of DNA during replication or in RNA during transcription. Describe the consequences that might result from a base pair substitution occurring in DNA and RNA.

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 are reported as the mean and standard error (+/-) of the mean 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.

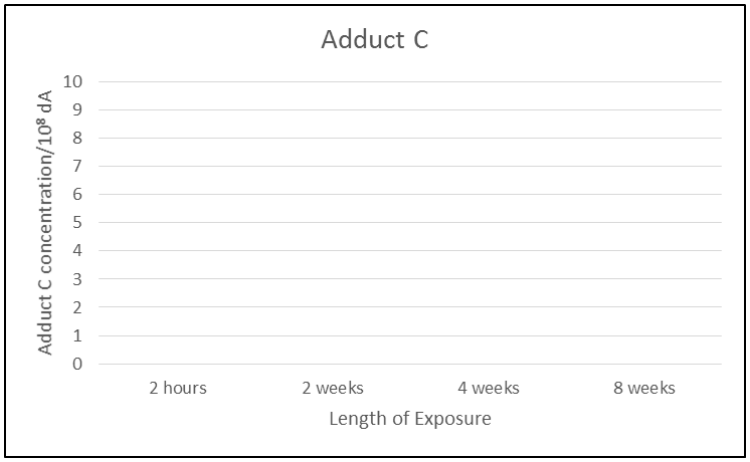
Time post-exposure	**Adduct A Concentration**		**Adduct B Concentration**		**Adduct C Concentration**	
	Adduct A /10^5 G	($^{13}C_2$) Adduct A /10^5 G	Adduct B /10^8 G	($^{13}C_2$) Adduct B /10^8 G	Adduct C /10^8 dA	($^{13}C_2$) Adduct C /10^8 dA
	Endogenous	*Exogenous*	*Endogenous*	*Exogenous*	*Endogenous*	*Exogenous*
2 Hours	0.2 ± 0.1	10.4 ± 2.3	4.1 ± 2.8	18.9 ± 4.9	4.9 ± 0.6	5.1 ± 0.6
2 Weeks	0.1 ± 0.03	0.4 ± 0.3	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: