



# 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.

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## 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 line 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 trichloroethylene (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 <sup>2</sup> ,3-ethenoguanine (Adduct B)	εG	Promutagenic activity during DNA synthesis	150 days
1,N <sup>6</sup> -ethenodeoxyadenosine (Adduct C)	εdA	Promutagenic activity during DNA synthesis	~1day

*\*These adducts are modified nitrogenous bases. For the purpose of this activity, the scientific names of these adducts aren't important but students should understand that these are modified nitrogenous bases in DNA nucleotides. 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.*

These DNA adducts are produced in different amounts, with 7-OEG (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 (<sup>13</sup>C<sub>2</sub>)-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** (EdA) 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.**

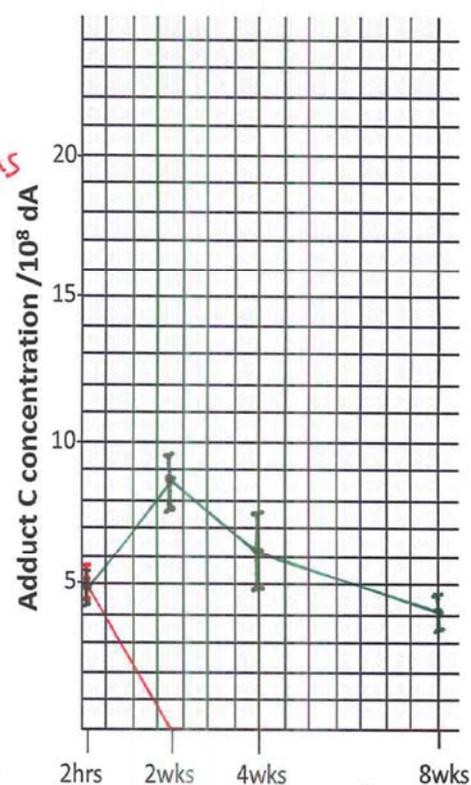
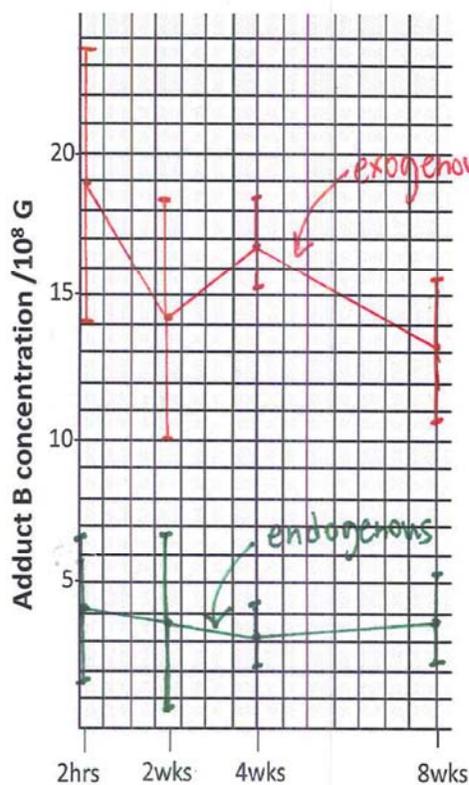
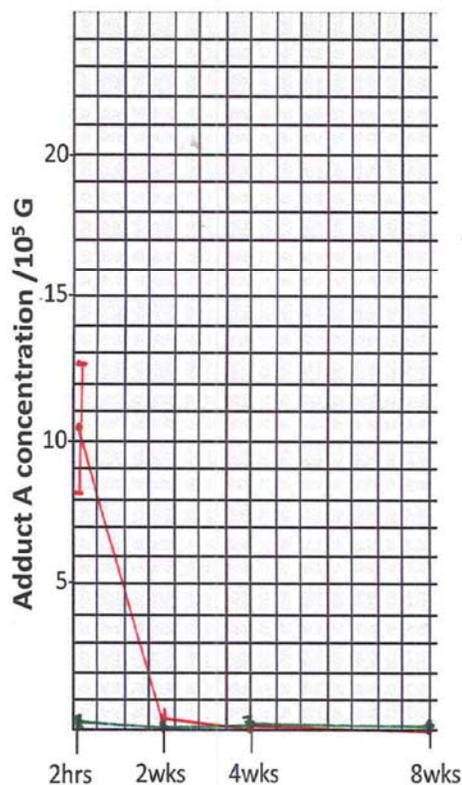
You could conclude this activity by discussing the possible consequences of a DNA adduct not being repaired. A DNA adduct that is not repaired can result in the insertion of an incorrect base (base-pair substitution) in the opposite DNA strand during DNA replication or in its complementary RNA strand 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. 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! 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.
2. Distinguish between endogenous and exogenous exposure to chemicals and discuss the relevance in relation to chemical exposure (improved risk assessment, cancer, etc).
3. 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*).
4. Introduce students to the research of Jim Swenberg, DVM, PhD, at UNC-Chapel Hill. 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/>
5. 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.
6. In the case of vinyl chloride (VC), it 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 them these four adducts can also occur inside cells under normal metabolic conditions in the absence of VC exposure.

- 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 ( $^{13}\text{C}_2$ ) VC to distinguish between endogenously derived adducts and adducts arising from exogenous exposure to VC (see *Teacher PPT slides 8-9*). Students will examine data (see *Teacher PPT slides 10-11*) 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; EG: Mutlu, 2010; EdA: Gao, unpublished)
- 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.
- Next, tell students they will complete Part II of their worksheet. **Orient students to the data table in part II before instructing them to construct line graphs for Adducts A, B and C.** 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.
- 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*.



- Review the answers to the questions in Part II as a class; completed worksheets may be collected for assessment.
- 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.

## Acknowledgements

This lesson was created by Dana Brown Haine, MS, of the UNC Superfund Research Program (SRP), which is funded by the National Institute of Environmental Health Sciences (P42ES005948). This lesson was reviewed by SRP researcher Jim Swenberg, DVM, PhD. This lesson was thoughtfully reviewed and piloted by AP Biology and AP Environmental Science teacher Donald R. Kirkpatrick of Marion High School in Marion, South Carolina.

## Resources

J. Nakamura, Esra Mutlu, Vyom Sharma, Leonard Collins, Wanda Bodnar, Rui Yu, Yongquan Lai, Benjamin Moeller, Kun Lu, James Swenberg. *The endogenous exposome*. DNA Repair 19 (2014) 3–13.  
<http://www.sciencedirect.com/science/article/pii/S1568786414001025>

Mutlu E, Jeong YC, Collins LB, Ham AJ, Upton PB, Winsett D, Hatch G, and Swenberg JA. 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/>

Mutlu E, Collins LB, Stout MD, Upton PB, Dave LR, Winsett D, Hatch G, Evansky P, Swenberg JA. 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/>

Pottenger LH1, Andrews LS, Bachman AN, Boogaard PJ, Cadet J, Embry MR, Farmer PB, Himmelstein MW, Jarabek AM, Martin EA, Mauthe RJ, Persaud R, Preston RJ, Schoeny R, Skare J, Swenberg JA, Williams GM, Zeiger E, Zhang F, Kim JH. *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. 2014 Apr;44(4):348-91.  
<http://www.ncbi.nlm.nih.gov/pubmed/24494825>

### The Exposome

C.P. Wild. *Complementing the genome with an “exposome”: the outstanding challenge of environmental exposure measurement in molecular epidemiology*. Cancer Epidemiol. Biomarkers Prev., 14 (2005), pp. 1847–1850  
<http://cebp.aacrjournals.org/content/14/8/1847.full.pdf+html>

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

C.P. Wild. *The exposome: from concept to utility*. Int. J. Epidemiol., 41 (2012), pp. 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/>

Vinyl Chloride, ToxFAQs, ATSDR  
<http://www.atsdr.cdc.gov/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](http://www.nature.com/nrc/journal/v4/n8/fig_tab/nrc1410_F2.html)

**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 to 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 line graphs for Adducts A, B and C. Once you have completed each 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.*

**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 to determine the impact of exogenous exposure of VC on DNA adduct formation.

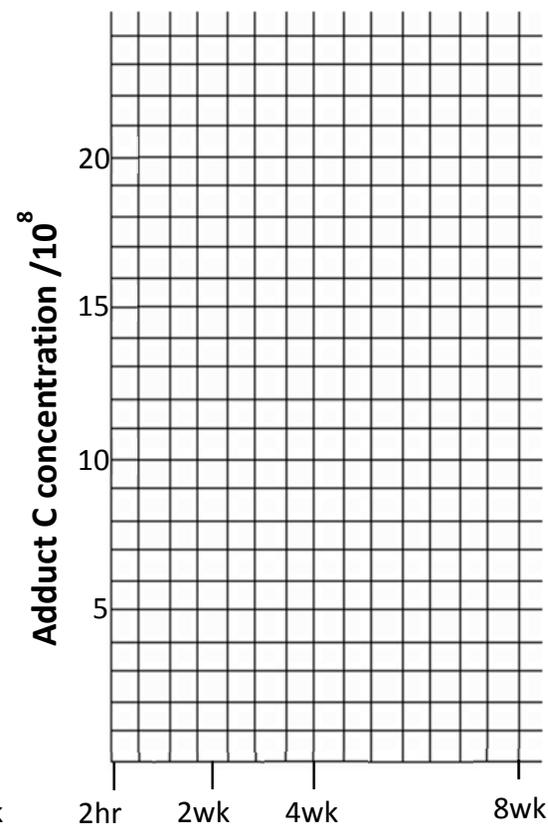
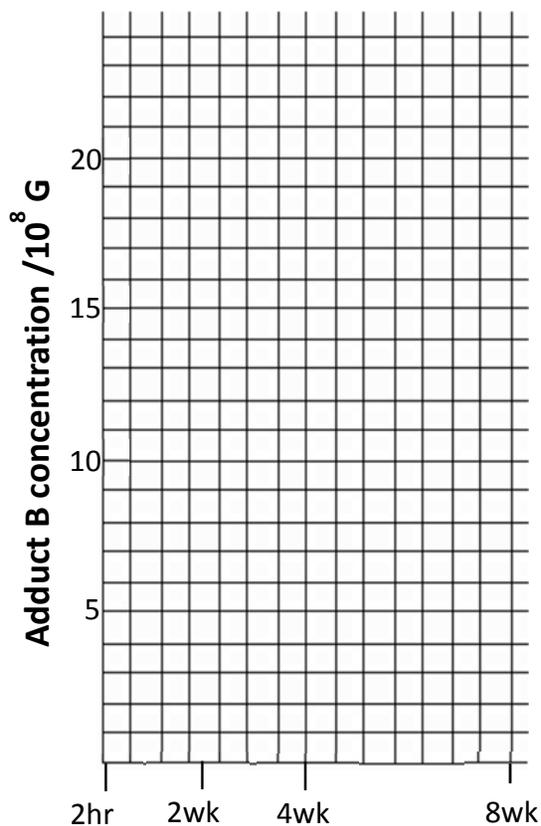
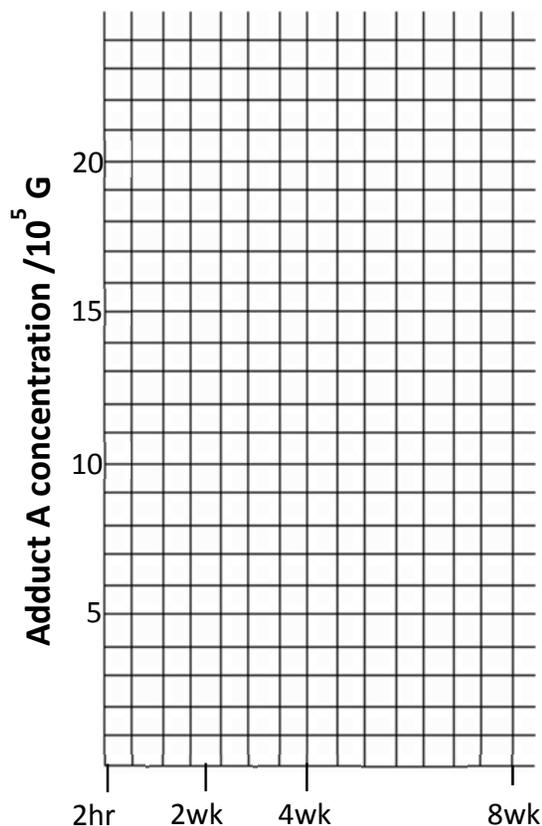
**Part II.**

**Use the data on the back of this sheet to construct line graphs for Adducts A, B and C. Once you have completed each 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.

Use the data below to determine the extent to which endogenous and exogenous DNA adducts form and the extent and speed to which adducts get repaired. To distinguish between endogenous and exogenous adducts, scientists can use stable isotope labeled ( $^{13}\text{C}_2$ ) VC and track its incorporation into DNA adducts in rodents. Scientists exposed rats to air containing 1100 ppm ( $^{13}\text{C}_2$ )-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 ( $^{13}\text{C}_2$ ) VC will have a higher mass than adducts produced by exposure to endogenous chemicals. **For each adduct below, construct a corresponding line graph using the data points provided; each graph will have two lines, one showing endogenous adducts and the other exogenous adducts.** It is fine to connect the dots just remember that there are error bars for each data point; label each line. The units here are  $10^5$  G,  $10^8$  G or  $10^8$  dA which is simply the number of adducts for every  $10^5$  or  $10^8$  guanine (G) or adenine (dA) nucleosides.

Time post-exposure	Adduct A Concentration		Adduct B Concentration		Adduct C Concentration	
	Adduct A / $10^5$ G	( $^{13}\text{C}_2$ ) Adduct A / $10^5$ G	Adduct B / $10^8$ G	( $^{13}\text{C}_2$ ) Adduct B / $10^8$ G	Adduct C / $10^8$ dA	( $^{13}\text{C}_2$ ) Adduct C / $10^8$ dA
	<i>Endogenous</i>	<i>Exogenous</i>	<i>Endogenous</i>	<i>Exogenous</i>	<i>Endogenous</i>	<i>Exogenous</i>
2 hours	$0.2 \pm 0.1$	$10.4 \pm 2.3$	$4.1 \pm 2.8$	$18.9 \pm 4.9$	$4.9 \pm 0.6$	$5.1 \pm 0.6$
2 Weeks	$0.1 \pm 0.03$	$0.4 \pm 0.3$	$3.7 \pm 3.1$	$14.2 \pm 4.2$	$8.6 \pm 0.9$	Not detected
4 Weeks	$0.2 \pm 0.04$	$0.1 \pm 0.06$	$3.1 \pm 1.0$	$16.9 \pm 1.6$	$6.2 \pm 1.3$	Not detected
8 Weeks	$0.2 \pm 0.07$	Not detected	$3.7 \pm 1.5$	$13.2 \pm 2.5$	$4.1 \pm 0.5$	Not detected



Time post-exposure	Adduct A Concentration		Adduct B Concentration		Adduct C Concentration	
	Adduct A /10 <sup>5</sup> G	( <sup>13</sup> C <sub>2</sub> ) Adduct A /10 <sup>5</sup> G	Adduct B /10 <sup>8</sup> G	( <sup>13</sup> C <sub>2</sub> ) Adduct B /10 <sup>8</sup> G	Adduct C /10 <sup>8</sup> dA	( <sup>13</sup> C <sub>2</sub> ) Adduct C /10 <sup>8</sup> dA
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

