Research Exam Q7 – *Biomedical evidence in practice* (2018-2019)

April 10, 2019

During the exam, you have access on a computer to these books:

- Baynes & Dominiczak: Medical Biochemistry
- Campbell: Statistics at square one
- Donders: Literature Measurement errors
- Fletcher: Clinical Epidemiology
- van Oosterom en Oostendorp: Medische Fysica
- Petrie and Sabin: Medical Statistics at a Glance
- Turnpenny: Emery's Elements of Medical Genetics
- Casarett & Doull's Essentials of Toxicology (3e)
- Form with statistical formula's

You are allowed to use a calculator of the type Casio FX-82MS.

The questions must be answered in English. If you cannot remember a specific English term, you may use the Dutch term.

Write your name and student number on the first page of each question!

Name:

Student Number:

General question (Gert Olthuis)

Question 1 (6 points)

The Health Council provides advice to the government regarding public health matters. This involves complex decision making based on factual and normative (un)certainties. The text in the box below is taken from a recent report the Health Council published in collaboration with the COGEM (The Netherlands Commission on Genetic Modification): *Editing Human DNA. Moral and social implications of germline genetic modification.*

In this report the Health Council of the Netherlands and COGEM describe the technical, legal and ethical issues raised by human germline modification. The main questions examined are:
• What is known about the effectiveness and safety of germline genetic modification in the short and long term, both for individuals and for society as a whole? What research is needed to clarify these issues?
• What is the legal and ethical framework for germline genetic modification? What aspects of the existing legal and ethical framework are being stretched by current developments in gene technology?
• How can the government, professional groups and society steer the governance of germline modification in an acceptable direction?

The table below allows the classification of the problem of allowing human germline modification that is discussed in the report, based on certainty pertaining normative criteria and the knowledge about the underlying health issue.

	HIGH certainty on knowledge	LOW certainty on knowledge
HIGH consensus on normative criteria	A	B
LOW consensus on normative criteria	C	D

Which cell (A, B, C or D) of this table provides the best classification of the problem of human germline modification, as presented in the box above? Explain your answer with one argument regarding the certainty of knowledge and one argument regarding the consensus on normative criteria surrounding this issue.

Correct cell (4 pt): D.

Argument on normative criteria (3 pt): The questions make clear that the issue of human germline modification puts pressure on the ethical framework. This technical development raises new ethical issues on which there is no consensus in society.

Argument on knowledge (3 pt): The first question in the box makes clear that there is a need to know more about the effectiveness and safety of germline modification. Certainty with regard to knowledge on long term effects is low.

Name:

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Vaccination (Saskia van Selm)

Question 2 (9 points)

Rubella is a viral illness that can cause a slight fever in children and a mild rash that can last for three days. However, when Rubella infection occurs during pregnancy, serious consequences can result, including miscarriages, fetal deaths/stillbirths and severe birth defects known as Congenital Rubella Syndrome. Vaccination with the mumps-measles-rubella combination vaccine (MMR, or BMR in Dutch) is highly effective in preventing infection with Rubella virus and gives lifelong protection. The estimated vaccine coverage to reach herd immunity is 85%.

a. In a previously not vaccinated population, Rubella vaccination is introduced in a childhood vaccination program. What would be the consequence of vaccination with a suboptimal coverage of 50% On Rubella disease in the next 2-3 decades? (4 points)

Incidental outbreaks. Part population is protected by natural infection or vaccination. But also a part is not immune and not all of these individuals will be .

b. What is the effect on the incidence of Congenital Rubella Syndrome? Explain your answer. (5 points)

Increase: more pregnant women are not immune at child-bearing age (before vaccination almost everybody was immune by natural infection)

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Vaccination (Saskia van Selm)

Question 3 (10 points)

Vaccination against influenza is recommended from the age of 60 years old, since this population is vulnerable to serious complications of the disease. This vaccine can be obtained via the general practitioner.

You have developed a universal influenza vaccine that is broadly protective against all influenza strains. Preclinical trials and Phase I and II trials have shown very promising results, so it is decided to move ahead and try to get this vaccine registered. To do so, a randomized clinical trial has been performed with 6000 elderly between 60-70y randomized across two arms. The first arm will receive the newly developed influenza vaccine, whilst the other group will receive a control vaccine against pneumococcal infections (i.e. not protective against influenza). After follow-up during the winter season. 2% of the group given the new influenza-vaccine developed influenza. In the group that received pneumococcal vaccination, 20% developed influenza.

	Vaccinated group	Control group (not vaccinated)
Influenza cases	60 (2%)	600 (20%)

a) Calculate the vaccine efficacy. (3 points)
(0.2-0.02)/0.2=0.9 90%

b) You want to register this vaccine. Name the four most important criteria that are used by the Health Council in forming an advice whether to introduce the vaccine. (4 points)

Disease Burden for individual patient
Effectiveness in prevention disease or complications
Safety: no serious health effects that substantially redce the individual health gain
Efficiency: favourable ratio cost and effects relative to other interventions

c) Ten years later you want to know if the vaccinated persons are still protected or if they are at risk to develop disease and need to be immunized again. Name an alternative method that you can use to predict protection in this population without waiting for disease as the clinical endpoint for protection? Explain when this is a valid prediction. (3 points)

If a correlate of protection is known, you can measure this in e.g. in blood

Name:

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Risk assessment (Frans Russel)

Question 4 (10 points)

In Europe, a number of medicinal products containing the active substance valsartan were recently recalled. There is a risk that certain batches may have been contaminated with the carcinogenic substance N-nitrosodimethylamine (NDMA) by a change in the production process. Medicines containing the active substance valsartan are used to treat high blood pressure and heart failure. NDMA is classified as a probable human carcinogen. This means that exposure over the long term could increase the risk of cancer.

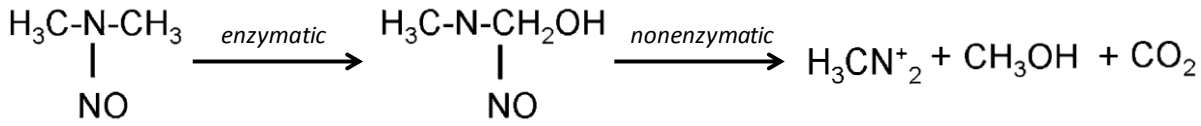
The amounts of NDMA present in the valsartan active ingredient varied, but on average were higher than levels that are considered reasonably safe. The valsartan active ingredient contained on average 60 parts per million (ppm) NDMA and the exposure was approximately 3 years. In Table 1, estimates were derived of the possible increased cancer risk.

Table 1. Estimated potential increased cancer risk for patients taking various doses of NDMA-containing valsartan products for 3 years

Valsartan dose (mg/day) containing 60 ppm NDMA	**Risk estimate**
40	1 additional case per 93,400 people
80	1 additional case per 46,700 people
160	1 additional case per 23,300 people
320	1 additional case per 11,600 people

For patients taking the highest dose of valsartan (320 mg) containing 60 ppm (= 19.2 µg) NDMA per tablet once daily for three years, the potential increased risk of cancer over a lifetime could be 1 additional case of cancer for every 11,600 people taking the product.

a. The biotransformation of NDMA in the body consists of enzymatic degradation into hydroxymethylnitrosamine, which in turn is nonenzymatically converted into methyldiazonium, methanol and carbon dioxide.



NDMA hydroxymethylnitrosamine methyldiazonium

What type of reaction is represented by the enzymatic step? Which family of metabolizing enzymes is responsible for this type of conversion? (2 points)

b. Which molecule is likely carcinogenic: NDMA, hydroxymethylnitrosamine, or methyldiazonium? Explain your answer. (2 points)

c. In an exposure study in dogs the following toxicokinetic parameters were found for NDMA: oral bioavailability (F) = 90%, total body clearance (CL) = 40 mL/min/kg, apparent volume of distribution (V) = 2 L/kg body weight. Assume that these parameters are also valid for human exposure, do you expect NDMA to accumulate in the body of a patient taking 320 mg/day valsartan? Motivate your answer. (2 points)

d. Suppose a patient had taken 80 mg/day valsartan over a period of 30 years, what would be the potential increased risk of cancer over a lifetime for this person. (2 points)

e. Put the risk you estimated (from question d) into a broader context, given that the maximal tolerable lifetime risk to exposure of environmental carcinogens is 1:10,000 and that nearly 1 in 2-3 people are expected to develop cancer during their lifetime. Do you agree with the recall of the valsartan tablets or do you consider it an exaggerated measure? Motivate your answer. (2 points)

Answers

a. *Oxidation (Phase I), cytochrome P450 family*
b. *Mehyldiazonium, is a highly reactive alkylating intermediate that damages proteins and DNA.*
c. *NDMA will not accumulate because the half-life is very short compared to the dosing interval of 24 hrs; t1/2 = ln2*V/CL = 0.693*2 L/0.04 L/min = 35 min.*
d. *Risk is 1:46,700 for 3 years 80 mg valsartan once daily. This can be extrapolated linearly to a period of 30 years, resulting in a lifetime risk of 1:4,670.*
e. *The risk is considered minimal in the context of the lifetime risk of spontaneously developing cancer, but relatively high as compared to the maximal tolerable risk set for environmental exposure. Since the contamination is avoidable by going back to the old production process and patients are dependent on this medication, it is the right decision to have these batches recalled and oblige the manufacturers to change the production to a process with undetectable impurities of NDMA.*

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Risk assessment (Frans Russel)

Question 5 (10 points)

A farmer in Ulestraten (Limburg) is growing Kanzi apples at close distance to the runway of Maastricht-Aachen Airport. He is worried about kerosene fuel deposit on his product. The apple grower sent some of his apples to a laboratory for analysis of Mineral Oil Saturated Hydrocarbons (MOSH). The MOSH content was 160 mg/kg. According to the European Food Safety Agency (EFSA) the Acceptable Daily Intake (ADI) for mineral oils is of 12 mg/kg bodyweight/day for mineral oils (EFSA 2012). The ADI is defined as is a measure of the amount of a specific substance in food or drinking water that can be ingested (orally) on a daily basis over a lifetime without an appreciable health risk (WHO, 1987).

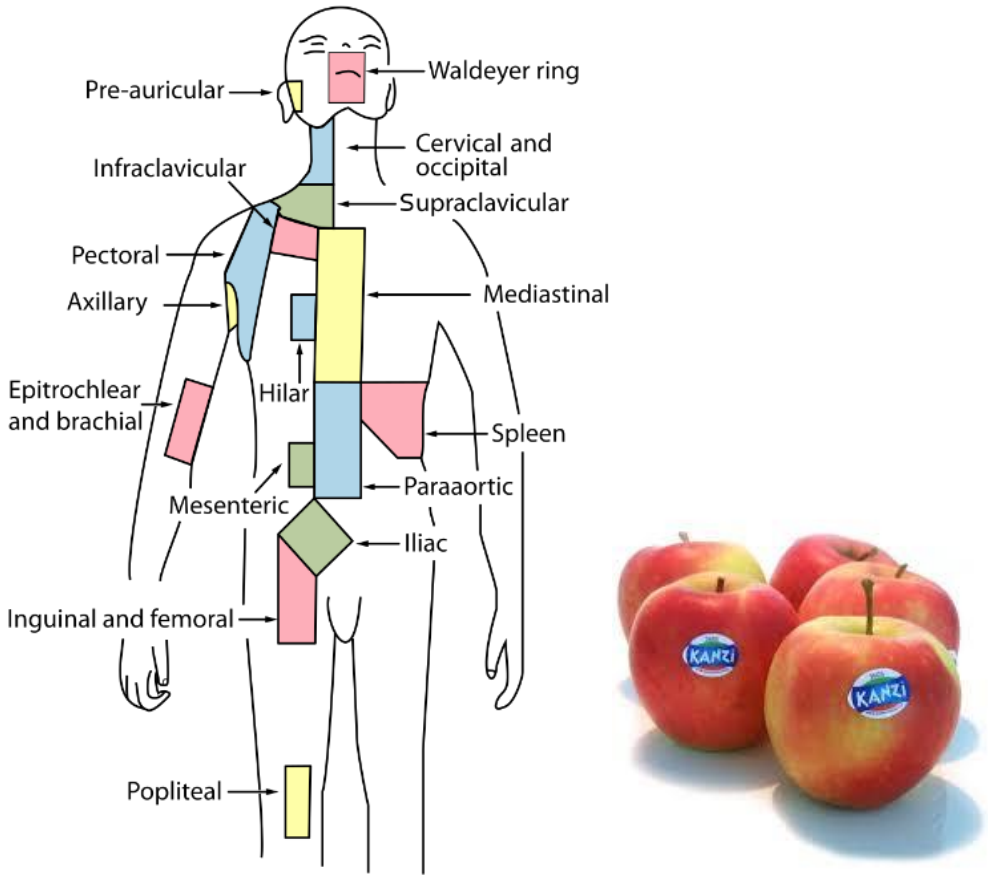

***Figure 1**. Mesenteric lymph nodes MLN) are located in the stomach (left). One kg of Kanzi apples (right).*

Read the following excerpt of the concluding section from the EFSA report (2012) and provide answers to the questions below:

> *In rats, the available data for high molecular weight mixtures showed effects related to MOSH accumulation in the liver and in mesenteric lymph nodes (MLN). Fischer 344 rats showed sensitivity to the development of liver and MLN granulomas and microgranulomas following the subchronic exposure to mineral oils and waxes. The presence of microgranulomas/histiocytosis in MLN was considered a nonspecific, adaptative change of low toxicological concern. In the liver, however, the microgranulomas were surrounded by inflammatory cells. Evidence from a two-year carcinogenicity study on two high viscosity mixtures (P70(H) and P100(H)) suggest that the microgranulomas in liver and MLN do not cause a prolonged inflammatory response or other severe pathological changes following chronic exposure in Fischer rats (Trimmer et al., 2004). However, it was assumed that liver microgranulomas, observed in the Fischer 344 rats, could be potentially relevant to humans and therefore the critical effect for the risk assessment of MOSH.* [Source: EFSA report (Mineral oil hydrocarbons in food. EFSA Journal 2012;10(6):2704 131)]

a. What does EFSA define as the critical effect in the Fischer rats? Do you agree with this choice? Motivate your answer. (3 points)

b. What are the two most important sources of uncertainty that should be taken into account when the rat data are used to derive an ADI? (2 points)

c. How many apples can a person eat before the ADI is reached? You may assume that an apple has an average weight of 200 g and that the body weight of an average person is 70 kg. (3 points)

d. What should the farmer tell a mother who buys an apple at the farm to give to her 6-year-old daughter? (2 points)

Answers

a. *EFSA adopts hepatic microgranulomas as the critical effect. They describe this endpoint also in MLN as 'nonspecific, adaptative change of low toxicological concern' so not a clear adverse effect. However, somehow similar histopathology seen in the liver is considered critical, apparently because inflammation is observed, which is considered to be adverse but no severe pathology was observed in a chronic rat study. The committee states that they still consider these 'critical' effects are 'relevant to humans'. This is not a much convincing statement and will be difficult to defend to risk assessors.*
b. *The most important sources of uncertainty in the extrapolation of animal data to humans are intraspecies differences and interspecies differences.*
c. *For an adult of 70 kg the maximum daily dose is 70 x 12 = 840 mg. This amount corresponds to 840/160 = 5.25 kg of apples. One kg corresponds to 5 apples. So, a person can eat 5 x 5.25 = 26.26 apples before the reaching the ADI.*
d. *Overall, eating an apple is considered a beneficial to health. The ADI will protect her child from any adverse health effect, as far as we know, even if you take into account a much lower body weight of a 6-year old. Because the contamination is probably only on the surface the farmer could just give the general advice to wash the apple with water from the tap to remove any dust/contaminants before consumption.*

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Screening as early diagnostics (Jos van Dijck)

Question 6 (15 points)

Rengaswamy Sankaranarayanan and colleagues reported on their study about the long term effect of screening for oral cancer with visual inspection in a randomized trial in Kerala, India [Oral Oncology. 2013; 49: 314-21].

In the group that was invited for the screening, 49,179 healthy individuals age 35 years and above did attend the 1st round to get a physical examination by visual inspection of their oral cavity conducted by trained health workers. Some of the screening outcomes are presented in Table 1.

Table 1 – Outcomes of the first round of screening for oral cancer by visual examination of the oral cavity.

	Oral cancer		
Visual examination	yes	no	total
Positive	79	3,510	3,589
Negative	x	y	45,590
	79 + x	3,510 + y	49,179

The performance of a screening test can be evaluated according to its test properties.

a. The sensitivity of the screening test cannot be calculated because essential information is missing. What missing information is necessary? (2 points)
b. Calculate the positive predictive value (%) and the oral cancer detection rate (‰). (2 points)
c. Based on these figures, is visual inspection a meaningful screening test to detect oral cancer? (6 points)

d. In people chewing tobacco or smoking bidi and cigarettes in addition to drinking alcohol, a much higher prevalence of oral cancer was noted compared to persons who did not use tobacco or alcohol. (Bidi is a small hand-rolled cigarette made of tobacco and wrapped in a leaf.)
If screening with visual inspection would have been applied to this high-risk group, what do you expect to happen to the positive predictive value and false-positive rate of the screening test? Explain your answer. (5 points)

Answers

To Q6

a. To calculate sensitivity, the number of false-negatives (x) must be known. In case of screening, this means the number of cancers detected in screen-negatives before the next round of screening. This usually requires linkage to a cancer registry and/or follow-up of the people with a negative screening outcome.
b. PV+ = 79/3589 = 2% whereas detection rate = 79/49179 = 1.6 ‰
c. PV+ of 2% is very low, meaning that of all people with a positive screening test, 98% will end up being false-positive.
   Detection rate is low as well. Possibly the sensitivity of visual inspection is low, meaning cancers may be missed by the screening test.

d.

- PV+ will be higher because underlying oral cancer incidence is higher.
- However, if focused on the bidi and cigarette smoking population, the oral cavity will most likely be discoloured, show subtle lesions, or show inflammation. This will probably yield many false-positive results.
- In the bidi and cigarette smoking population, the oral cavities will appear to look discoloured and inflammated. The risk of a false-positive screening result is probably higher, and specificity lower.