Research Exam Q7, biomedical sciences – 2016-2017

April 5, 2017

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

Casarett & Doull's Essentials of Toxicology (3e);

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.

Name: **Studentnummer:** | | | | | | | |

Screening

Question 1 - Colorectal cancer screening (17 pt)

Colorectal cancer is an important health burden. In the Netherlands colorectal cancer was diagnosed in more than 15.000 individuals (2015) and about 5.000 people died from this disease (2014). In 2012 the 5-year survival was between 62 and 65%. The rationale behind colorectal cancer screening is that a cancer develops from adenomatous polyps. These polyps, especially when bleeding microscopically, are fairly easy to find and remove. There are various tests available that could be considered for use as a screening test, see table below (based on table 4 from the Dutch Health Council Report on colorectal cancer screening, published November 2009).

Table - Expected outcomes for five screening tests in one screening round. Percentages.

Screening test	Sensitivity for advanced adenomas	Sensitivity for colorectal cancer	PPV for advanced adenomas	PPV for colorectal cancer	Risk of complications without further assessment (colonoscopy)	Risk of complications including colonoscopy	Participation
Colonoscopy	>90	97	6,7	0,8	0,1	not applicable	20-25?
CT-colonography	>90	97	40-67, depending on cut-off	5-9, depending on cut-off	very small (probably <0,00005)	0,02	35?
gFOBT	12	20	41	10	0	0,006	47
iFOBT	27	65	40	8	0	0,017	60
Sigmoidoscopy	55	60	79	6	0,002	0,026	30

In 2011 the Minister of Health, Welfare and Sports decided to implement a national screening programme for colorectal cancer starting in 2014. The target group consists of men and women aged 55 to 75 years (about 4 million). This group will receive a biennial invitation to provide a stool sample that will be checked for traces of blood using the immunohistochemical Faecal Occult Blood Test (iFOBT). Traces of blood can be caused by colorectal cancer or polyps, but can also have other causes. The expected participation in screening at that time for iFOBT was 60%. Applying a cut-off value of 75 ng/ml it was expected that 6,4% of the screened persons would have a positive iFOBT. The sensitivity and specificity for the detection of colorectal cancer at the selected cut-off value are 65% and 97% respectively. All persons with a positive screening test will be offered further assessment in hospital.

The follow-up examination consists of an internal surveillance examination of the colon, known as colonoscopy. In preparation for the colonoscopy, the person is asked to drink a strong liquid laxative at home. The aim is to clean and empty the colon, so the stool is watery and almost clear. In most cases, pain and sleep medication (fentanyl, midazolam) is administered before the colonoscopy. The examination takes about 20 minutes. If possible, polyps are directly removed. If this is not possible, a small piece of tissue is removed (biopsy) and examined by the pathologist.

A. Provide two arguments why the Minister of Health, Welfare and Sports selected iFOBT as the preferred screening test. (4 pt)

- *de opkomst is hoger dan bij de andere tests*
- *het onderzoek is niet invasief, vraagt geen uitgebreide voorbereiding en kan thuis worden uitgevoerd (lage belasting)*
- *het onderzoek heeft een kleine kans op complicaties*

B. Based on the information provided above, how many persons on a yearly basis will be referred for further assessment in hospital on the basis of the iFOBT test? (4 pt)

60% van 4 miljoen per 2 jaar is 1,2 miljoen testen per jaar.
6,4% heeft een positieve screeningstest, dit zijn 76.800 personen.

C. Explain that the introduction of colorectal cancer screening can both increase and decrease the incidence of colorectal cancer. (4 pt)

- *toename van incidentie is mogelijk door overdiagnose, dwz de diagnose van carcinomen die zonder screening nooit symptomatisch geworden zouden zijn*
- *afname van incidentie is mogelijk doordat het voorstadium van kanker, de poliepen, bij screening kunnen worden verwijderd*

D. Several years ago, a general practitioner took the initiative to offer his 1300 patients a blood-based test (FOBT) for colorectal cancer. This was before the Minster had decided to implement a screening programme in the Netherlands. The general practitioner argued: “Yearly, 4500 persons in the Netherlands die of colorectal cancer. So early detection is of paramount importance.” Do you agree with the general practitioner? Provide two arguments for or against. (5 pt)

- *Screening is niet van belang vanwege deze getallen. Er moet voldaan worden aan de criteria van Wilson en Jungner.*
- *Voorbeelden uit de criteria van Wilson en Junger, bijv het moet zijn aangetoond dat vroegere opsporing en behandeling effectiever zijn (minder sterfte aan die kanker) dan reguliere zorg, het moet kosteneffectief zijn, etc.*
- *Bovendien hebben we in Nederland een wet op Bevolkingsonderzoek die zegt dat je niet zo maar mag beginnen met een screeningsprogramma. Zonder vergunning mogen alleen mensen met klachten worden onderzocht.*

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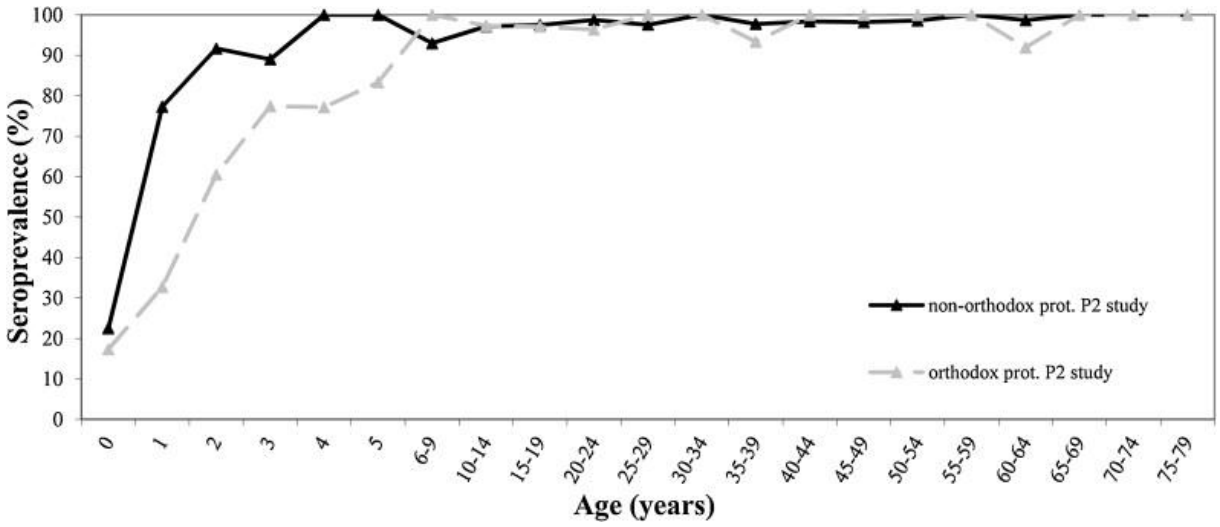
Vaccination

Question 2 – Rubella (10 pt)
Although rubella disease is typically mild, infection during pregnancy can result in miscarriage or congenital rubella syndrome (CRS). The rubella vaccine was introduced in the Netherlands in 1974, initially only for girls. Since 1987 all children are vaccinated against rubella. The graph below shows the weighted age-specific seroprevalence for rubella – the percentage of persons in a population with detectable antibodies specific to rubella virus – in Orthodox Protestant individuals (OPIs) and non-OPIs in an area with low vaccination coverage.



A. Explain the differences and/or similarities in seroprevalence at the age of 2 and 10 years of age between the OPI and the non-OPI group. (5 pt)

a. Age of 2y: Vaccination coverage in the OPI group is lower than in the non-OPI group, resulting in less individuals with detectable antibody levels against rubella
b. Age of 10y: due to reduced vaccination coverage rubella is still endemic in this population. This leads to natural infections over time and consequently an increase in the number of individuals with a positive antibody response increases gradually until almost all individuals have a detectable antibody response

B. Not reaching a critical vaccination threshold for infant rubella vaccination can have dramatic consequences. In this context, describe the changes in protection levels in women of child-bearing age over time (short term and long term) following implementation of a rubella childhood immunization program. (5 pt)

a. Short term: Vaccination will result in protection in vaccinated individuals. Most unvaccinated women of child-bearing age will remain partially protected due to having build up natural immunity following infection at a young age.

b. Long term: Due to suboptimal vaccination coverage rubella is still endemic and due to the gradual increase of susceptible individuals, this will ultimately result in an outbreak of rubella (and CRS)

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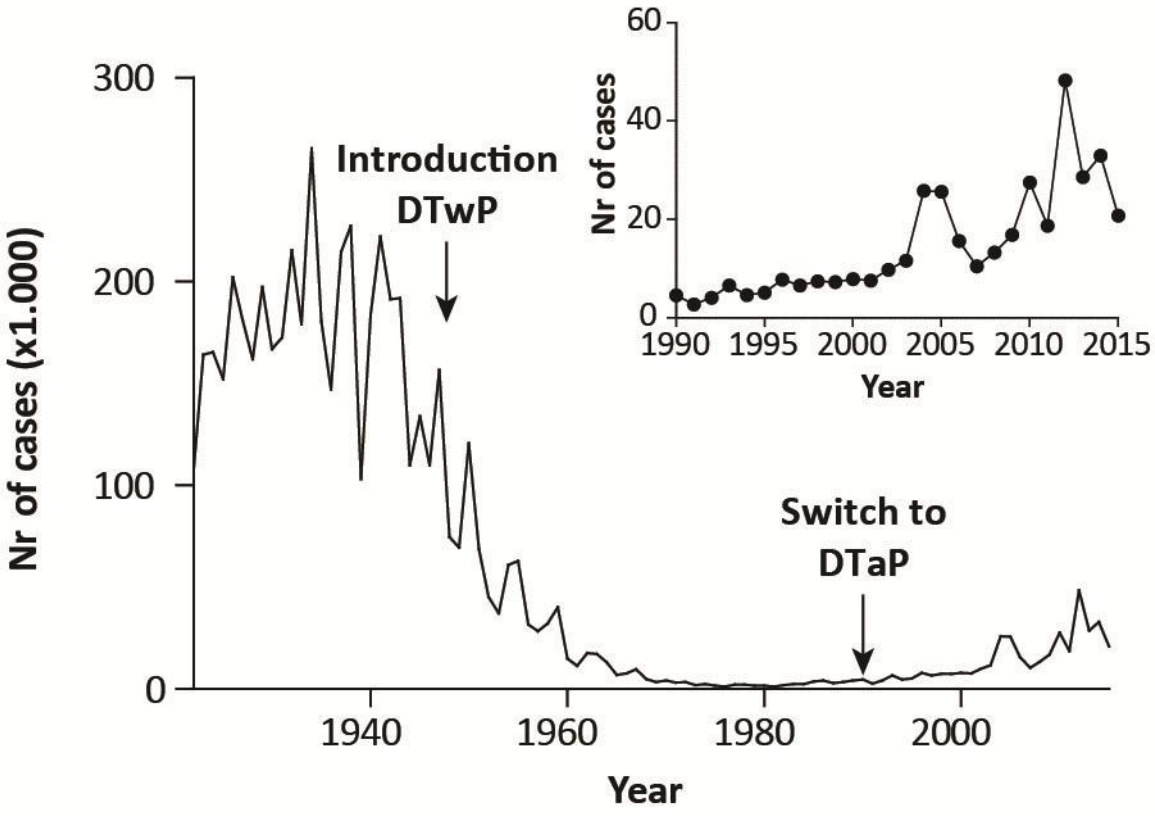
Question 3 – Pertussis (14 pt)

Both the whole-cell (wP) and the acellular pertussis vaccine (aP) provide protection against disease. The graph below shows the cases of pertussis in the US in the pre-vaccine area as well as after the introduction of the whole cell pertussis vaccine (DTwP) in the 1940s and the later switch to the acellular pertussis vaccine variant (DTaP). In the USA, the vast majority of children is vaccinated against pertussis.



A. For both the wP and the aP vaccine, describe the two major advantages (benefits) and two disadvantages (risks) in national immunization programs. (4 pt)

a. wP advantages: longer-lasting protection against infection, better herd immunity
b. wP disadvantages: side effects, reduced acceptance
c. aP advantages: less side effects, protection against disease
d. aP disadvantages: reduced duration of protection, less herd immunity, risk of

B. Various vaccine companies are actively developing new safe and improved pertussis vaccines. What are the two major objectives of a Phase III clinical trial with this vaccine? (4 pt)

Safety and efficacy

C. Explain two major difficulties regarding the design of a Phase III trial with such a vaccine in a country with a high vaccination coverage. (4 pt)

a. *Because the vast majority of the population is already vaccinated and protected against disease, there are relatively few cases each year and a Phase III efficacy trial would require huge numbers of subjects, possibly even the whole population. This would be extremely costly.*
b. *Because acellular vaccines already protect very efficiently against disease in the short term, a phase III trial would require a long follow-up to observe any protective effects of the vaccine.*

D. Describe one way how a correlate of protection could facilitate this process. (2 pt)

a. *Shorten trials by providing alternative immunological end-points*
b. *Making clinical trials smaller as the immunological end-point will theoretically be reached in all subjects, whereas a clinical end-point will only be reached in a small proportion of participating subjects*

Question 4 – Mechanisms of protection (6 pt)

Following injection of a vaccine, there are several essential steps in the immune response that ultimately result in the generation of antibodies and clinical protection. Describe the three cell types that are essential for antibody production and describe for each of these cell types in 1-2 sentences their function in this process.

1. *Antigen-presenting cells (uptake and presentation of antigens to T cells)*
2. *T cells (providing help to B cells)*
3. *B cells (activation by antigen and CD4 T cells result in production of antibodies)*

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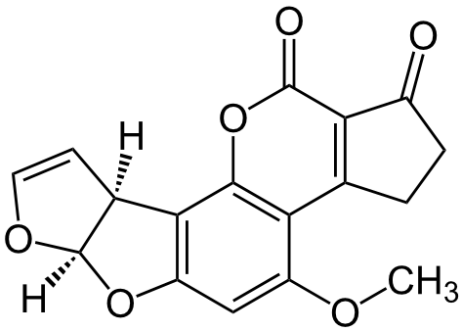
Risk assessment

Question 5 – Aflatoxine (15 pt)

Aflatoxin B1 is produced by *Aspergillus flavus* and is a very potent carcinogen implicated in hepatocellular carcinoma in humans. It is a common contaminant in a variety of foods including peanuts, corn, and other grains.



Aflatoxin B1

In 2009 the European Food Safety Authority (EFSA) concluded that public health would not be adversely affected by allowing the levels for total aflatoxins from 4 µg/kg to 10 µg/kg for all nuts. An increase in average total dietary exposure of approximately 1 % was estimated. This was based on a better characterization of the variability in the exposure assessment of aflatoxin.

A. What is the difference between variability and uncertainty in risk assessment? (3 points)

Variability refers to the inherent heterogeneity or diversity of data in an assessment (expressed as variance, standard deviation, etc.). Uncertainty refers to a lack of data or an incomplete understanding.

B. Name one variability factor and one uncertainty factor that could play a role in the exposure assessment of aflatoxin B1. (3 pt)

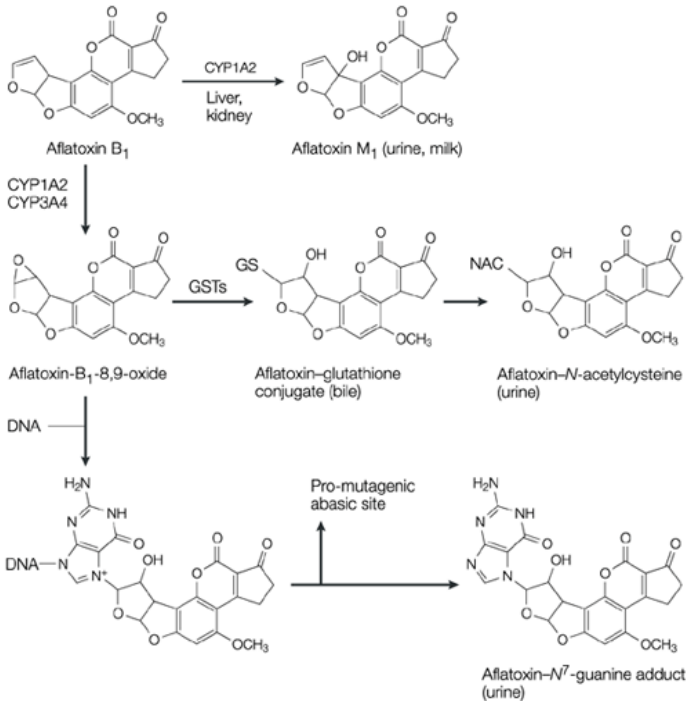
Variability: (1) measurements of source contaminant concentrations, (2) environmental parameters (e.g., pH, temperature, humidity), (3) human exposure factors (e.g., age, gender, eating behaviour, individual susceptibilities). Uncertainty: (1) descriptive errors, (2) incomplete analysis, (3) measurement or sampling errors, (4) exposure model uncertainty (e.g., errors, parameter uncertainty, incorrect model)

C. Preliminary toxicokinetic data of aflatoxin were obtained in a human volunteer study. After an oral dose of 31 ng an AUC of 7 pg.hr/L was found. What can you conclude about the oral bioavailability of aflatoxin: could it be 100% or was it much less? Motivate your answer. (5 points)

*CL = F*D/AUC, if F would be 100% CL = 31,000 (pg)/7 (pg.hr/L)= 4,428 l/hr. This is an unrealistically high CL, which theoretically cannot be higher than 300 L/hr (= cardiac output) and will be likely less than the liver plasma flow (40 L/hr). This means that the bioavailability will be low (<10%).*

D. Aflatoxin B1 requires metabolic conversion to its 8,9-epoxide in order to cause cancer. Draw the biotransformation product aflatoxin B1-8,9-oxide and explain on a molecular level how this reactive intermediate causes cancer. (4 points)

E.



Cytochrome P450 (CYP) family

Question 6 – Toxicity of Formaldehyde (18 pt)

Formaldehyde is used for fixation of human remains in anatomy and pathology. Students receive their anatomy training in a room where (parts of) human remains are used that are treated with 3-4 % aqueous solution of formaldehyde in water ('formaline'). This year the University of Utrecht decided that pregnant students are not allowed to participate in the anatomy training any more. This decision was based on a meta-analysis indicating an effect of formaldehyde exposure on the rate of spontaneous abortions (see below).

Reproductive and Developmental Toxicity of Formaldehyde: A Systematic Review

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Mutat Res. 2011 November ; 728(3): 118–138

Abstract

Formaldehyde, the recently classified carcinogen and ubiquitous environmental contaminant, has long been suspected of causing adverse reproductive and developmental effects, but previous reviews were inconclusive, due in part, to limitations in the design of many of the human population studies. In the current review, we systematically evaluated evidence of an association between formaldehyde exposure and adverse reproductive and developmental effects, in human populations and in vivo animal studies, in the peer-reviewed literature. The mostly retrospective human studies provided evidence of an association of maternal exposure with adverse reproductive and developmental effects. Further assessment of this association by meta-analysis revealed an increased risk of spontaneous abortion (1.76, 95% CI 1.20–2.59, p=0.002) and of all adverse pregnancy outcomes combined (1.54, 95% CI 1.27–1.88, p<0.001), in formaldehyde exposed women, although differential recall, selection bias, or confounding cannot be ruled out. Evaluation of the animal studies including all routes of exposure, doses and dosing regimens studied, suggested positive associations between formaldehyde exposure and reproductive toxicity, mostly in males. Potential mechanisms underlying formaldehyde-induced reproductive and developmental toxicities, including chromosome and DNA damage (genotoxicity), oxidative stress, altered level and/or function of enzymes, hormones and proteins, apoptosis, toxicogenomic and epigenomic effects (such as DNA methylation), were identified. To clarify these associations, well-designed molecular epidemiologic studies, that include quantitative exposure assessment and diminish confounding factors, should examine both reproductive and developmental outcomes associated with exposure in males and females. Together with mechanistic and animal studies, this will allow us to better understand the systemic effect of formaldehyde exposure.

The announcement of the University of Utrecht that pregnancy is a reason to expel students from participation in anatomy training is a big step. Especially because there is not yet a 'formaldehyde-free' alternative for this training. Based on the information provided, you are invited to give your expert opinion to the head of the Anatomy Department who is responsible for the anatomy training of students at Radboudumc.

A. How do you weigh the evidence and its relevance from human and animal data (as presented in the abstract)? Use ordinal scale for your rating of the weight-of-evidence: e.g. very weak, weak, moderate, strong, very strong. Also express whether or not you consider the evidence presented as *relevant* for the research question on reproductive outcome, use the rating 'high' or 'low'. Note that you have to make a decision concerning evidence from animal data and human data, separately. Motivate your expert opinion (8 pt)

The authors describe the association with reproductive and developmental effects in animal studies as 'suggested positive' and relate this health outcome to several existing and plausible biological mechanisms which supports that formaldehyde could be a reproductive toxic hazard obtained in an experimental setting using standardized conditions. Therefore, the evidence from animal data is considered 'strong' because these mechanisms are known toxicity pathways also in humans. However, the results were observed 'mostly in males'. This raises the question how relevant this information is to the issue of pregnancy. Also there is the uncertainty if such effects can be extrapolated from animals to humans. Therefore relevance to pregnancy is considered 'low'.

The human data were previously described as inconclusive. However, this systematic review from 2011 supports a possible association of formaldehyde exposure with a relevant reproductive outcome, but there are some uncertainties expressed by the authors (selection bias and confounding). The evidence of human studies is therefore weighed as 'moderate' but the relevance as 'high'.

B. What uncertainties do you see in the animal data and in the human data? Explain how they have an impact on uncertainty related to the dilemma of the anatomy training. (4 pt)

*For animal data the uncertainties three key issue related to **interspecies difference, inter-individual differences in susceptibility** and the **gender issue** because the dilemma relates to pregnancy (may effects seen in male animals be considered of relevance to females). Other issues that would normally play a role are extrapolation from **high-to-low dose and route-to-route extrapolation**. Note that the authors addressed both of these uncertainties and and have included these uncertainties in their overall statement ('suggested positive' rating).*
For human data it is clear that there may be methodological limitations related to the retrospective design of (most studies): recall bias (e.g. possible role of peak exposures), selection bias ('negative' studies may not have been published) and confounding (there are many other risk factors related to the endpoints studied (spontaneous abortion and pooled (!) developmental effects) that may be present in the studied populations

C. If the head of the Anatomy Department would like to continue using formaldehyde how could he/she use the precautionary principle to address the problem? Also explain how the precautionary principle could be implemented in practice. (6 points)

The precautionary principle would be 'better safe than sorry' and would leave a final decision not based on the scientific decision itself but to the individual (this is called a hedonistic approach in ethics). For implementation it would be possible to make the issue ('as-is') part of the anatomy training and provide the students with the human and animal data, including the uncertainties. It is important that the anatomy department and instructors do not draw their own conclusion (and make that public) but leave that to participants. The students could then decide for themselves if they have a personal reason not to be involved in practical anatomy. This arrangement has some advantages and limitations:
Advantages
- *It respects the student's right to privacy (concerning the pregnancy issue)*
- *Student that have a child-wish and have stopped the use of contraceptives could decide not be exposed, also during the first trimester of pregnancy, when the sensitivity is the highest.*

Limitations
- *It is much easier to say this is the problem, this is the solution and decide for the students (the top-down model).*