Exposure setups for in vivo experiments using waveguides

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1. Common features of the experimental design for three \textit{in vivo} studies and the deduced technical requirements

2. Technical implementation of completed studies with AKR/J - mice involving GSM and UMTS exposure at 900 and 2000 MHz, respectively

3. Running long-term UMTS exposure of mice at 2 GHz
   - design and modified set-up
   - field distribution
   - dosimmetrical investigations

4. Conclusion
Experimental design

- 3 studies in cooperation with the International University Bremen (Prof. A. Lerchl):

1. Influence of 900 MHz electromagnetic fields on spontaneous leukaemia in AKR/J mice
   160 mice exposed to generic GSM test signal, 400 mW/kg (whole body), 24 hrs / day

2. In vivo experiments on exposure to the high frequency electromagnetic fields of mobile telecommunication. B. Carcinogenesis
   same as 1., but generic UMTS test signal. End: 4/2005

3. Long-term study on the effects of UMTS signals on laboratory rodents
   Four generations of mice (c. 1200 animals altogether) exposed to generic UMTS test signal, different specific absorption rates (SAR). Endpoints: fertility, development, teratogenic effects. End: 7/2007

⇒ For all studies the biological design was prescribed by the Federal Office for Radiation Protection (BfS) concerning
   • exposure of freely moving animals
   • exposure at fixed averaged whole body specific absorption rates (SAR)
   • exposure by generic GSM900 or UMTS test signals
Exposure of non-restrained animals

• The prescribed biological design allows
  - a long-term exposure up to 24 hours per day
  - animals held in cages (i.e. usual laboratory environment)
  - standard animal handling

• but can also introduce variations of the exposure due to
  1. different locations of the animals in the incident field
  2. varying orientations (postures) of the animals with respect to the
direction of wave propagation and the polarization of the field vector
  3. the superposition of the original incident field and the scattered field
from neighbouring animals
  4. the age-dependent development of body mass

⇒ While points 2. + 4. are inherent to the biological design and can not be
avoided, the effects of 1. + 3. can be reduced by a suitable technical design
of the exposure device
Reduction of whole body exposure variation

• The technical design should provide a field distribution inside the cage as uniform as possible in order to minimize the variations due to different locations of the moving animals.

• Also, a well-defined stable and reproducible field distribution reduces the effect of scattered fields from neighbouring animals (e.g. the excitation of higher order modes)

These features together with the above mentioned requirement of a very high number of simultaneously exposed animals can be fulfilled with the concept of the radial waveguide.
Design of a radial waveguide

- parallel circular metal plates
- absorbing material at radial boundary (mandatory for experiments with free-running animals in order to minimize the SWR)
- feed system: rotational symmetrical cone antenna in the centre exciting radially propagating waves
- cages arranged at constant distance from the centre
Advantages of radial waveguide exposure chamber

+ simultaneous exposure of numerous biological samples

+ uniform exposure due to highly symmetrical configuration

+ high ratio of power density to generated power

+ electrically closed system
  ⇒ no additional shielding of laboratory
  exposed and sham-exposed waveguide can be located close to each other

+ complete numerical analysis feasible
  ⇒ modelling one sector with proper boundary conditions
  containing field excitation, exposed animals in the cage and
  termination of the waveguide
Exposure field: fundamental $\text{TM}_{00}^{z}$ (TEM) -mode

- waveguide’s height < half the wavelength
- rotational symmetrical excitation (empty waveguide) \{ only TEM-wave propagating

\[
E_z(\rho, \varphi, z) = -j A \frac{k_0^2}{\omega \mu_0 \varepsilon_0} H_0^{(2)}(k_0 \rho) \\
H_\varphi(\rho, \varphi, z) = -A \frac{k_0}{\mu_0} H_0^{(2)'}(k_0 \rho)
\]

- E-field polarized in vertical direction
- field strength constant in the waveguide’s cross-section ($\rho =$ const.)
- field decays for large radial distances $\sim \frac{1}{\sqrt{\rho}}$
  - for ordinary arrangements field decay in exposure region rather small
- Therefore, the TEM-mode is well suited for the generation of a uniform exposure field.
Higher order modes

If height of the cages that is prescribed by animal protection authority requires a waveguide’s height of \( h \geq \lambda/2 \)

\[ \Rightarrow \] higher order modes with respect to the vertical direction can propagate in addition to the TEM-mode

- **TE_{01}^z**-mode:

\[
E_\varphi (\rho, \varphi, z) = A_{01} \frac{k_\rho}{\varepsilon} \frac{H_0^{(2)}}{H_0^{(2)}}(k_\rho \rho) \sin \left( \frac{\pi}{h} z \right) \\
H_\rho (\rho, \varphi, z) = -j A_{01} \frac{k_\rho k_z}{\omega \varepsilon \mu} H_0^{(2)}(k_\rho \rho) \cos \left( \frac{\pi}{h} z \right) \\
H_z (\rho, \varphi, z) = -j A_{01} \frac{k_\rho^2}{\omega \varepsilon \mu} H_0^{(2)}(k_\rho \rho) \sin \left( \frac{\pi}{h} z \right)
\]

- **TM_{01}^z**-mode:

\[
E_z (\rho, \varphi, z) = -j B_{01} \frac{k_\rho^2}{\omega \mu \varepsilon} H_0^{(2)}(k_\rho \rho) \cos \left( \frac{\pi}{h} z \right) \\
E_\rho (\rho, \varphi, z) = j B_{01} k_\rho \frac{k_z}{\omega \mu \varepsilon} H_0^{(2)}(k_\rho \rho) \sin \left( \frac{\pi}{h} z \right) \\
H_\varphi (\rho, \varphi, z) = -B_{01} \frac{k_\rho}{\mu} H_0^{(2)}(k_\rho \rho) \cos \left( \frac{\pi}{h} z \right)
\]

Higher order modes have non-uniform field distribution in the waveguide’s cross section and different propagation properties compared to the TEM-mode.
Single mode excitation of cage region

\[ \lambda /2 < h < \lambda \]

- fundamental \( \rightarrow \) mode +
- higher order \( \rightarrow \) modes

\[ \Rightarrow \text{exposure field: superposition of all propagating modes} \]

\[ \Rightarrow \text{can lead to non-reproducible exposure (interference effects) due to different propagation properties} \]

\[ \Rightarrow \text{higher order modes must be suppressed} \]
Single mode excitation of cage region

counter measure: reduction of waveguide’s height for smaller radii

⇒ single mode excitation of cage region
Example: single mode excitation of the cage region

- frequency 2 GHz
- cage height 16 cm (h = \(\lambda\))
- height in the cage region: 17 cm
  height in the centre: 6 cm

⇒ uniform field distribution in vertical direction, exposure field is given by the TEM-wave

- unwanted field components in the cage region are negligible as compared to the wanted z-component of the fundamental mode
Exposure of large animals or groups of animals

- For animal or group size in the dimension of wavelength the scattered field is not negligible and can affect the field distribution in neighbouring cages.
- Due to the movement of the animals the excitation of higher order modes with circumferential variation can lead to non-reproducible exposure.
- Therefore a ‘decoupling’ of adjacent cage regions is necessary in order to keep stable exposure field conditions.
**Approach A:**

decoupling of adjacent sectors by metal bars

- metal bars attached to the upper and lower plate between the cages
- bars do not alter the propagation properties of the TEM-mode
- but shift-up the cut-off frequencies of unwanted higher order modes out of the frequency range of operation
- height \( H \) and width \( W \) optimised by numerically solving the eigenvalue problem of the waveguide
- applicable for waveguide heights \( \lambda/2 \leq h \leq \lambda \) and exposure of large animals or larger numbers of mice per cage
Example: field distribution in cross-section of sector decoupled by metal bars

empty sector with metal bars:
exposure field given by TEM-wave only

sector with metal bars, cage and simplified models for animals (ellipsoids):
- expected field distortions (scattered fields) caused by animals are restricted to the immediate vicinity of the animals
- neighbouring sectors are decoupled
Approach B: decoupling of adjacent sectors by 'high impedance' walls

- application for waveguide heights $h \geq \lambda$ and exposure of large animals or larger numbers of mice per cage
- 'high impedance' walls must not alter the propagation properties of the TEM-mode
- therefore, the wall impedance must tend to infinity $Z_w \to j \propto$
Cross section of sector with high impedance walls

corrugated metal walls with dielectric inserts $\varepsilon_r$
grooves are oriented in parallel to the propagation of the TEM-wave $\rho$-direction

$$Z_W = \frac{E_z}{H_\rho} \bigg|_{y=b-t_{\text{opt}}} = j Z_0 \mu_r \sqrt{\frac{\mu_r}{\varepsilon_r}} \sqrt{1 - \left(\frac{m\lambda}{2w}\right)^2} \tan \left( \frac{2\pi t_{\text{opt}}}{\lambda} \sqrt{1 - \left(\frac{m\lambda}{2w}\right)^2} \right) \rightarrow j \infty$$
with
$$t_{\text{opt}} = \frac{\lambda}{4} \sqrt{\frac{1}{\varepsilon_r \mu_r} - \left(\frac{m\lambda}{2w}\right)^2}^{-1}$$

anisotropic wall impedance tends to infinity for the optimum groove depth
Model for sector with high impedance walls

- **PMC (boundary condition)**
- **canonical transition region**
- **absorber**
- **TEM-wave**
- **dielectric material (green)**
- **grooves oriented in parallel to propagation of the TEM-wave (yellow)**
Computed field distribution with high impedance walls

\[ f = 2 \text{ GHz} \]

\[ Z_W \rightarrow j\infty \]

⇒ uniform field distribution in vertical and azimuthal direction

⇒ exposure field only given by the TEM-wave
Technical implementation

• 3 studies in cooperation with the International University Bremen (Prof. A. Lerchl):

1. **Influence of 900 MHz electromagnetic fields on spontaneous leukaemia in AKR/J mice**
   160 mice exposed to generic GSM test signal, 400 mW/kg (whole body), 24 hrs / day

2. **In vivo experiments on exposure to the high frequency electromagnetic fields of mobile telecommunication. B. Carcinogenesis**
   same as 1., but generic UMTS test signal. End: 4/2005

3. **Long-term study on the effects of UMTS signals on laboratory rodents**
   Four generations of mice (c. 1200 animals altogether) exposed to generic UMTS test signal, different specific absorption rates (SAR). Endpoints: fertility, development, teratogenic effects. End: 7/2007

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   • exposure by generic **GSM900 or UMTS** test signals
Exposure of AKR/J mice to GSM 900 test signal

- Life-long exposure
- 7 mice per cage (cage height 15.5 cm)
- 24 exposed / 24 sham-exposed cages
- Exposure device:
  2 radial waveguides with diameter of 4 m and height of 17 cm ($\lambda/2 \leq h \leq \lambda$),
  24 sectors decoupled by metal bars

Results already published

GSM 900: exposure system (1/2)
GSM 900: exposure system (2/2)
Electric field and SAR distribution

Example of electric field distribution for different configurations of 7 mice per cage

SAR distribution in detailed mouse model with spatial resolution of $(1.2 \text{ mm})^3$, model scaled from a 7-day-old rat (IT’IS, Switzerland)

Averaged whole body $\text{SAR}_{\text{wb}}$ of 0.4 W/kg at $P_{\text{in}} = 35$ W

Standard deviation of $\text{SAR}_{\text{wb}}$: $\sigma \approx \pm 40\%$

The evaluation of organ-specific SAR is not necessary due to the biological endpoints, but the SAR distribution must be checked with respect to possible ‘hot spots’.
Technical implementation

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   - exposure of **freely moving** animals
   - exposure at **fixed** averaged whole body specific absorption rates (SAR)
   - exposure by generic **GSM900** or **UMTS** test signals
Exposure of AKR/J mice to UMTS test signal

- Same exposure condition as for the GSM 900 study, but height of cages reduced from 15.5 cm to 7 cm

- Exposure device: same radial waveguides as for the GSM 900 study, but reduced height of 8 cm ($\frac{\lambda}{2} \leq h \leq \lambda$)

Results already published


UMTS: 2 GHz exposure system
Electric field and SAR distribution

Example of electric field distribution for different configurations of 7 mice per cage

SAR distribution in detailed mouse model with spatial resolution of $(1.2\, \text{mm})^3$, model scaled from a 7-day-old rat (IT'IS, Switzerland)

Averaged whole body $\text{SAR}_{\text{wb}}$ of 0.4 W/kg at $P_{\text{in}} = 15$ W

Standard deviation of $\text{SAR}_{\text{wb}}$: $\sigma = \pm 50\%$

$\text{SAR}_{\text{wb}} = 253$ mW/kg at $P_{\text{in}} = 15$ W

The evaluation of organ-specific SAR is not necessary due to the biological endpoints, but the SAR distribution must be checked with respect to possible 'hot spots'.
Technical implementation (3)

- 3 studies in cooperation with the International University Bremen (Prof. A. Lerchl):

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  - exposure of freely moving animals
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  - exposure by generic GSM900 or UMTS test signals
Exposure of strain C57BL mice to UMTS test signal (running project)

- 2688 animals are exposed during the study
- 128 cages in total:
  - 32 sham-exposed groups / 3x 32 groups exposed with 0.08, 0.4 and 1.3 W/kg

Flowchart of animals per cage:

1. 2 pregnant mice & 1 male mouse
   - 14 days

2. 1 pregnant mouse & 1 male mouse
   - 7 days

3. 1 female mouse (again pregnant), 1 male mouse & 6 pups
   - 21 days

4. 4 young mice per cage
   - 48 days
Exposure setup (1/2)
Exposure setup (2/2)
Field measurements in the implemented exposure device

normalized averaged field values for one cage

σ ≈ 25%
Animal models (1/2)

- 2 models obtained from IT‘IS, Zurich, Switzerland
  - 1\textsuperscript{st} model: pregnant rat: 275g, spatial resolution 1mm
  - 2\textsuperscript{nd} model: 7-day-old rat: 17g, spatial resolution 0.5mm

Adult mice (weight between 24g and 34g):
- Scaled from 1\textsuperscript{st} model: spatial resolution 1.2 mm
- Scaled from 2\textsuperscript{nd} model: spatial resolution 0.7 to 1.2mm

Young mice 17g:
- Scaled from 2\textsuperscript{nd} model: spatial resolution 0.5 mm

Mouse pup (age: 9 days):
- Ellipsoids were used: weight: 7 g;
- spatial resolution: 0.6 mm
Animal models (2/2)

E-field polarization parallel to the main body axis

- adult mouse
- pup
- young mouse

Graph showing the relationship between normalized wb SAR and weight for different animal models.
### Electrical material parameters

<table>
<thead>
<tr>
<th>material</th>
<th>permittivity</th>
<th>conductivity</th>
<th>tissue density</th>
</tr>
</thead>
<tbody>
<tr>
<td>tail/muscle(parallel_fiber)</td>
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<td>1,5417</td>
<td>1040</td>
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<td>tail/skin(dry)</td>
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<td>1,2517</td>
<td>1158,85</td>
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<td>body/air</td>
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<td>0,075</td>
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<td>body/tongue</td>
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<td>body/bone_cortical</td>
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<td>1300</td>
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<tr>
<td>avg. Rat tissue</td>
<td>43,195</td>
<td>1,411</td>
<td>1137,22</td>
</tr>
</tbody>
</table>

Material parameters according to Brooks database were used:

[http://www.fcc.gov/fcc-bin/dielec.sh](http://www.fcc.gov/fcc-bin/dielec.sh)

Uncertainty of the material parameters in literature:

- Changing the material parameters ± 10%
The decision which constellations of mice in a cage from an infinite number of possibilities are considered for a representative numerical dosimetry is not trivial, since

- the movement of the animals is a dynamic, non-deterministic process
- some constellations are more typical than others
- even small variations of a configuration can change the SAR distribution
- the number of computer runs must be limited to a certain quantum (e.g. < 100)
Ideally, many calculations and a weighting of different configurations according to their occurrence might be reasonable.

However, for a reliable statistic the required data are not available.

For a practicable assessment of the whole body SAR without weighting
- typical configurations were identified in agreement with the IU Bremen (e.g. agglomerating mice, pubs close to the dam)
- some configurations known as “worst cases“ were included in order to avoid an underestimation of SAR (e.g. mice erected on the hind legs, isolated animals)

For future investigations a registration of time-varying configurations is planned, but an exhaustive data collection in an animal laboratory must be assigned to a separate project.
The following configurations of mice in a cage were analysed:

<table>
<thead>
<tr>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Phase 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Adult mouse / young mouse / pup lengthwise in the cage" /></td>
<td><img src="image2.png" alt="Adult mouse / young mouse / pup across in the cage" /></td>
<td><img src="image3.png" alt="Adult mouse / young mouse / pup in an upright position" /></td>
<td></td>
</tr>
</tbody>
</table>

- **Phase 1**:成人老鼠/小老鼠/幼崽纵向在笼子里
- **Phase 2**:成人老鼠/幼崽横向在笼子里
- **Phase 3**:在直立位置的成年老鼠/小老鼠/幼崽
- **Phase 4**:成年老鼠/小老鼠/幼崽在笼子里的其他位置
1\textsuperscript{st} stage: exposure field for one configuration

- low field inside the biological objects
- higher field strength above the tail of the mice
- standing waves
  - due to reflections at the mice
  - due to reflections at the non-perfect absorber
1\textsuperscript{st} stage: whole body SAR for 3 mice per cage

For more than 1 animal, a simple estimation of the wb-SAR as a function of the location of the models is not always possible.
2nd stage: SAR distribution of 2 adult mice

example of 1 configuration

SAR distribution for 1 configuration

2nd mouse is shadowed by the first one

- larger SAR values occur in the 1st mouse

Wb SAR:

1st mouse: 1.18 W/kg

2nd mouse: 0.75 W/kg

P$_{\text{sector}}$ = 1W
2nd stage: whole body SAR for 2 mice per cage

![Graph showing SAR in W/kg for different configurations.](image)

- High dose, $P_{in} \approx 28W$
- $1.44 \text{ W/kg}$
- $\sigma \approx 28\%$
3rd stage: field distribution and SAR distribution (1/2)

an example of 1 configuration with 8 mice

exposure region

P_{sector} = 1W

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Reinhardt, Bitz, El Ouardi, Streckert, Hansen
3rd stage: field distribution and SAR distribution (2/2)

High field values at the outer surface of the animal, do not necessarily lead to high SAR values at the animals surface.

Field distribution of the 1st mouse

Wb SAR: 1.39 W/kg

Psector = 1 W

logarithmic scale
3rd stage: whole body SAR for 8 mice per cage

Whole body SAR for the adult mice

Whole body SAR for 6 pups (7g)

TEM

high dose, $P_{in} \approx 28$ W

$\sigma \approx 50\%$

$1.21$ W/kg

$1.92$ W/kg

$\sigma \approx 54\%$
4th stage: 4 young mice per cage; SAR distribution for one mouse

example of 1 configuration

wb.-SAR of the 1st mouse: 1.45 W/kg
Averaged wb-SAR for the configuration: 1.6 W/kg

\[ P_{\text{sector}} = 1W \]
4th stage: whole body SAR for 4 young mice per cage

![Graph showing SAR in W/kg for different configurations.]

- High dose, $P_{in} \approx 28W$
- $\sigma \approx 33\%$
- 2.34 W/kg
Conclusion

• With the modified radial waveguide setup we achieved a well defined exposure, merely leaving those variations of the whole body SAR introduced by the biological design.

• The prescribed SAR values of 1.3 W/kg, 0.4 W/kg, 0.08 W/kg were adjusted according to the 1st stage of the study (adult mice). The corresponding input power was kept constant during all stages because of the animals' rapid growth.

<table>
<thead>
<tr>
<th>PIN = 28 W; S = 22 W/m²</th>
<th>Wb -SAR for high dose in W/kg</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; configuration</td>
<td>1,3</td>
<td>31%</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; configuration</td>
<td>1,44</td>
<td>28%</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; configuration; adult:</td>
<td>1,21</td>
<td>50%</td>
</tr>
<tr>
<td>pups:</td>
<td>1,92</td>
<td>54%</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; configuration</td>
<td>2,34</td>
<td>33%</td>
</tr>
</tbody>
</table>